SEAGRASS (*ZOSTERA MARINA)* HEALTH IN A EUTROPHIC COASTAL MARINE ECOSYSTEM AS AFFECTED BY MULTIPLE ENVIRONMENTAL STRESSORS: SHADING, SEDIMENT ORGANIC MATTER, TOTAL SULFUR, AND SOLUBLE SULFIDES

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of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

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ABSTRACT

Increased organic matter inputs in eutrophic marine coastal systems may lead to high levels of microbial sulfate reduction and elevated concentrations of sulfide in porewaters, resulting in subsequent declines in seagrass health. I examined this process in West Falmouth Harbor, a nitrogen (N)- enriched lagoon in Cape Cod. West Falmouth Harbor can be divided into three sub-basins: Snug Harbor, which is impacted by N-inputs from groundwater and contained seagrass until a die-off event in 2010; the Middle Harbor, which is impacted by N-enrichment and contains seagrass; and the Outer Harbor, which contains a seagrass meadow but is less N-impacted. I found the highest levels of porewater sulfide in the Middle Harbor, with an average rooting zone value of 2.3 mM total soluble sulfide, as well as sediment organic matter values as high as 15%, and average $\delta^{34}S$ leaf tissue value of 0.0 ‰, implying plant exposure to isotopically light porewater sulfides. This contrasts with much lower sulfide and sediment organic matter concentrations in the Outer and Snug Harbors, at 1.0 and 0.7 mM for sulfide, and 6% and 8% organic matter, respectively. Soluble sulfide and $\delta^{34}S$ values found in 2007 in the innermost, highly eutrophic Snug Harbor sub-basin prior to the seagrass die-off event in 2010 were comparable to 2018 Middle Harbor values, with soluble sulfides at 3.0 mM and leaf tissue $\delta^{34}S$ at 0.2 ‰. Additionally, carbon values have decreased in Snug Harbor, from 4.4% in 2010 to 3.6% in 2018. I found indications of poor seagrass health in the Middle Harbor, including belowground biomass averaging 64 g per m^2, compared to 106 g per m^2 in the Outer Harbor. The Middle Harbor had a normalized difference vegetation index value nearly 2-fold higher than the Outer Harbor, indicating potential light limitation which would decrease seagrass photosynthesis and make them vulnerable to sulfide intrusion. A considerable amount of the light limitation experienced by seagrass in West Falmouth Harbor may result from epiphyte cover, with up to 0.55 mg epiphyte per cm^2 seagrass leaf area in both the Middle and Outer Harbors. My study suggests a feedback cycle in West Falmouth Harbor wherein sediment trapping of organic matter in N-enriched, light-limited conditions may highly stress seagrass's rhizome and root structure, leading to increased susceptibility to other environmental stressors and eventual mortality.

BIOGRAPHICAL INFORMATION

Katherine Haviland was born in Chicago, Illinois, and moved to Bel Air, Maryland shortly thereafter, where she spent her childhood enjoying the wetlands and tributaries of the upper Chesapeake Bay. She attended public school in Harford County, MD, and then went on to attend the University of Maryland, College Park, to achieve a B.S. in Geographical Sciences. While studying at the University of Maryland (UMD), she was fortunate to receive an opportunity conduct an undergraduate research project modeling and mapping the future impacts of climate change on flood levels and submerged aquatic vegetation health at Otter Point Creek, a tributary of the Bush River on the Chesapeake Bay, with the Anita C. Leight Estuary Center and the Chesapeake Bay National Estuarine Research Reserve (CBNERR). She first gained interest in biogeochemistry while working at Otter Point Creek, as she witnessed scientists with CBNERR conduct an analysis of the tributary's sediments. Following this, she decided to minor in Hydrology through UMD's geology department and gained further insight and interest in the field of biogeochemistry. She co-authored a paper on alterations in urban biogeochemistry with professors and graduate students in the department of Geology. Katherine then spent time working at Minidoka National Wildlife Refuge in Idaho, serving as a field assistant analyzing soils and wetland extent on the sagebrush steppe along the Snake River. After returning to Maryland, Katherine was employed as a lab assistant by the Paleoclimate Co-Laboratory on campus at UMD, assisting with stable isotope analysis of ^{13}C and ^{18}O in tree ring samples, eventually helping to train other students on isotope ratio mass spectrometry standard operating procedures. In 2017, she received the Anderson Award for excellence in the Geographical Sciences, awarded to the top undergraduate student in the Dept. of Geography. Later that year, she graduated summa cum laude from the University of Maryland and began attending Cornell University, pursuing an MS/PhD with the Howarth-Marino Lab through the graduate field of Natural Resources.

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I have been extremely fortunate to receive a huge amount of help and advice on this work from members of my lab group, including my advisor, Bob Howarth, as well as Roxanne Marino, and Melanie Hayn. Their input and guidance through the methods and analyses described as this paper were immensely valuable, and this work could not have taken place without them. I would like to specifically thank Bob for his support throughout the writing process, navigating the graduate school landscape, as well as in the lab assisting with sulfur biogeochemical analyses. I'm grateful for the considerable amount of time and effort Bob put into helping me understand the processes ongoing at our study site. I thank Roxanne for her advice and expertise in assisting me with laboratory techniques that were new to me, as well as helping with the writing process, and Melanie for her integral role in helping with the normalized difference vegetation index (NDVI) analysis, helping with SCUBA operations, and assisting with data management and methods testing throughout this process. I would like to thank all three of them for carrying out the 2005-2017 seagrass leaf tissue $\delta^{34}S$ observations which occurred before I joined the lab and were extremely valuable to my study. I'm also immensely grateful for their work on the NSF-funded Biocomplexity project, which provides important context for the work I present in this study. We've been fortunate to have several undergraduate assistants help us in the past two years, including Caroline Dodd, Nathalie Griffiths, and Vera Gaddi, all of whom assisted us with general laboratory work and were vital to the functioning of our research during the hectic field season. Other members of my lab group assisted me along the way, as well, including Michelle Wong, who helped me through writing successful grant proposals, and Dennis Sweney and Tom Butler for providing valuable feedback on my work in lab meetings. I'm also extremely appreciative of my committee member, Ian Hewson, for providing valuable feedback, advice, and direction for my work throughout the past two years.

I would also like to thank our colleagues at the Ecosystems Center at the Marine Biological Laboratory in Woods Hole, MA: Anne Giblin for assisting with SCUBA operations, and for leading the

2007 porewater sulfide analysis of Snug Harbor along with Clara Funk; Sam Kelsey for assisting with SCUBA operations and LECO sediment total sulfur analysis; and Marshall Otter for running our seagrass leaf tissue sulfur isotope samples through isotope ratio mass spectrometry. Several labs at Cornell have been integral to my research, as well, including the Goodale lab in assisting me with running my CNS sediment samples, and the Soil & Water Lab for helping me to run my sulfate and chloride samples.

I'm extremely grateful for funding sources that help support my work, including the National Science Foundation's Graduate Research Fellowship Program, Cornell Ecology and Evolutionary Biology (E&EB) Summer Research Fund, the Cornell Dept. of E&EB, the Cornell Department of Natural Resources, and Cornell College of Agriculture and Life Sciences Andrew W. Mellon foundation; as well as the National Science Foundation's Long Term Research in Environmental Biology program that supports our lab's work in West Falmouth Harbor.

I especially want to thank my loving parents, who've always supported me and my goal of pursuing marine ecosystem science and provided me the foundation I need to succeed.

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LIST OF ABBREVIATIONS

WFH	West Falmouth Harbor
N	Nitrogen
C	Carbon
S	Sulfur
NDVI	Normalized difference vegetation index
NIR	Near-infrared
LOI	Loss on ignition
ANOVA	Analysis of variance
MBL	Marine Biological Laboratory
SCUBA	Self-Contained Underwater Breathing Apparatus
PVC	Polyvinyl-chloride
CNS	Carbon-Nitrogen-Sulfur
DW	Dry weight
μM, mM	Micromolar, millimolar

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Abstract

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light limitation experienced by seagrass in West Falmouth Harbor may result from epiphyte cover, with up to 0.55 mg epiphyte per cm^2 seagrass leaf area in both the Middle and Outer Harbors. My study suggests a feedback cycle in West Falmouth Harbor wherein sediment trapping of organic matter in N-enriched, light-limited conditions may highly stress seagrass's rhizome and root structure, leading to increased susceptibility to other environmental stressors and eventual mortality.

Introduction

Nutrient pollution from terrestrial sources is a prominent feature of temperate estuaries, with more than 60% of U.S. coasts experiencing eutrophication as a result of nitrogen (N) pollution (Howarth et al., 2000). Eutrophication is often linked to the loss of seagrasses (Waycott et al., 2009). Under eutrophic conditions, seagrasses are subject to multiple environmental stressors, including elevated levels of porewater soluble sulfides and shading by increased epiphyte loads, resulting in poor health and mortality (Holmer, 2019). Hydrogen sulfide, which is often present at high levels in anoxic sediments, is a cytochrome c oxidase inhibitor and is toxic to seagrasses (Cooper & Brown, 2008). Seagrass can persist in high-sulfide, anoxic sediments due to passive diffusion of oxygen (O_2) from their roots as well as internal sulfide detoxification using photosynthetically derived O_2 (Hasler-Sheetal & Holmer, 2015). However, seagrass photosynthetic capacity declines in low-light conditions (Mochida et al., 2019), leaving them with lower levels of root oxygen leakage, and therefore vulnerable to soluble sulfide invasion (Brodersen et al., 2015). Another phenomenon occurring in light-limited conditions is an increase in seagrass chlorophyll content as an adaptive response to light scarcity (Ralph et al., 2007). Light availability and soluble sulfides may also influence seagrass biomass allocation, and ratios of above-ground to below-ground seagrass biomass have been used as a predictor of survivability in eutrophic conditions with higher values associated with poor survival outlook (Nixon et al., 2001). Declines in root and rhizome biomass may precede plant mortality due to high energy expenditure and O_2 demands from respiration of root and rhizome structures (Hemminga, 1998).

Seagrass meadows usually occur in sediments where organic matter composes less than 6% of sediment dry weight (Hemminga & Duarte, 2000). Seagrasses are adept at trapping particulate matter from the water column, and organic matter concentrations in seagrass meadow sediments are often much higher than in comparable un-vegetated locations (McGlathery et al., 2012), with increases of 2-fold commonly observed (Gacia et al., 2002; van Katwijk, 2010). In addition to sediment trapping, seagrasses and their epiphyte primary production further elevates sediment organic matter concentrations (Boschker et al., 2000). Seagrass canopy height also influences sediment conditions; the presence of seagrass increases the height of the diffusive boundary layer above the sediment-water interface and slows the diffusion of oxygen from the water column to the sediment surface (Koch et al., 2007; Jorgensen & Revsbech, 1985).

In eutrophic conditions, sediments may become highly enriched in organic matter due to increased sedimentation of biomass produced in the water column, as well as increased benthic production (Nixon, 1995). Sediment total organic matter in a eutrophic basin can be 3- to 4-fold greater than a nearby meso-oligotrophic basin (Dell'Anno et al., 2002). Increased sediment organic matter generally leads to increased benthic metabolism (Ferguson et al., 2003). In marine sediments, the reduction of sulfate to sulfide is an extremely important metabolic pathway—sulfate is highly concentrated in seawater, making it the major electron acceptor in estuarine sediments—and comprises as much as 70-90% of the microbial respiration in the sediments of productive estuaries (Howarth, 1984). Much of this sulfide precipitates as iron monosulfide (FeS) and as pyrite (FeS_2) (Howarth, 1984; Howarth & Jorgensen, 1984; Kraal et al., 2013). At high concentrations of porewater sulfide such as those found in eutrophic conditions, precipitation of FeS and FeS_2 may slow as Fe becomes less available, leading to elevated levels of sulfide in the porewaters (Giblin & Howarth, 1984). Which mineral precipitates depends on the redox conditions and pH of the porewaters, with FeS_2—the less soluble, more oxidized form—likely dominating in estuarine sediments (Luther et al., 1982).

Dissimilatory sulfate reduction leads to isotopic fractionation, with the sulfide end-product depleted in the heavier isotope, ^{34}S, relative to the sulfate being reduced. Seawater sulfate has an average $\delta^{34}S$ of +21 ‰ (Böttcher et al., 2007; Rees et al., 1978), while porewater sulfide $\delta^{34}S$ values in sediments underlying *Z. Marina* meadows often range between -22 to -30 ‰ (Frederiksen et al., 2006). In sulfate-depleted sediments, sulfide may diffuse into seagrass through their roots and rhizomes and move through lacunae structures to leaf tissue (Frederiksen et al., 2008). Seagrass tissue sulfur isotopes can be an indirect measure of the level of sulfide the plants are exposed to, with *Z. marina* shoots in high sulfide conditions demonstrating isotopically lighter $\delta^{34}S$ values than plants in low sulfide conditions (Fraser & Kendrick, 2017). Porewater sulfide has been found to be the source of as much as 68% of the sulfur found in leaf tissues growing on highly-organic sediments (Holmer & Hasler-Sheetal, 2014).

Seagrass meadows trap organic matter from the water column by increasing drag on and reducing the buoyancy of particles entering the seagrass meadow, as well as increasing the size of the boundary layer between the sediment surface and the water column. Seagrass particle trapping may be beneficial in nutrient-scarce, oligotrophic conditions, but can be detrimental in eutrophic basins where water column organic matter is high. I hypothesize that in eutrophic conditions, this trapping of fine particles leads to highly organic sediments and therefore high levels of porewater soluble sulfide, with negative effects on seagrass health (Fig. 1). I investigated this process along a gradient of N-enrichment in West Falmouth Harbor (WFH), a shallow estuary in Massachusetts, USA.

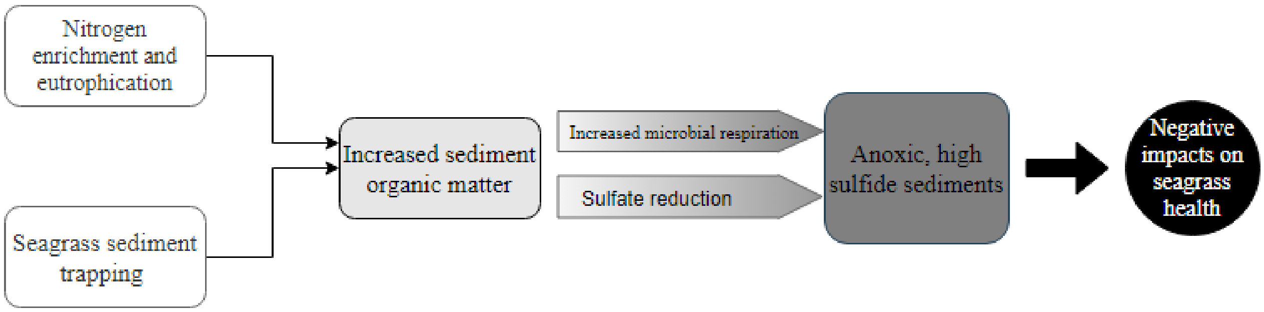

Fig. 1: conceptual model of our hypothesized system in West Falmouth Harbor.

Methods

Study site

My study site is WFH, a shallow lagoon (average depth at mean high tide is 1.9 m) with mean water residence time of 1 day (Howes et al., 2006), adjoining Buzzards Bay on Cape Cod, Falmouth, Massachusetts, USA. For a description of WFH see Hayn et al. (2013), and Howarth et al. (2014). WFH has received elevated N inputs from an aquifer contaminated by a wastewater treatment plant upstream of the lagoon since the early part of this century. The N from this contaminated aquifer enters largely through the Snug Harbor portion of WFH, as shown in Fig. 2 (Howarth et al., 2014). WFH receives an N load of ~4.2 mmol N m^{-2} day^{-1} from other watershed and atmospheric sources (Hayn et al., 2013). The Howarth-Marino Lab and our colleagues at the Marine Biological Laboratory (MBL) in Woods Hole, MA have collected water column nutrient, sediment, and seagrass data on 24 sites in WFH annually since 2005.

Between 2000 and 2010, subtidal eelgrass meadows were present in three sub-basins within WFH (Snug Harbor, Middle Harbor, and Outer Harbor) (Hayn, 2012). In 2010, seagrass meadow covered 20% of the sediments in Snug Harbor at mean water, before succumbing to a mortality event in July 2010 (Howarth et al., 2014; Hayn, 2012). As of June 2018, 0% of Snug Harbor, 60% of the Outer Harbor, and 68% of Middle Harbor sediment area were colonized by seagrass at mean water, with slight northward expansion of meadow area in the Middle Harbor basin occurring between 2010-2017 (Hayn et al., unpublished data).

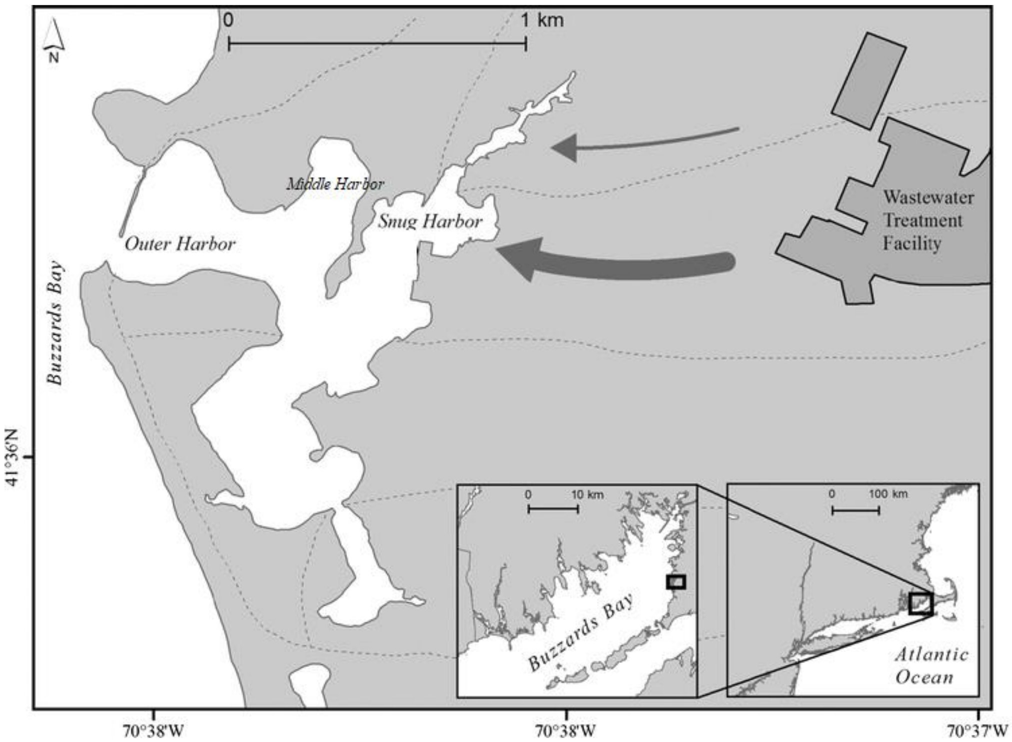

Fig. 2: Nitrate inputs to West Falmouth Harbor. Figure adapted from Howarth et al., 2014.

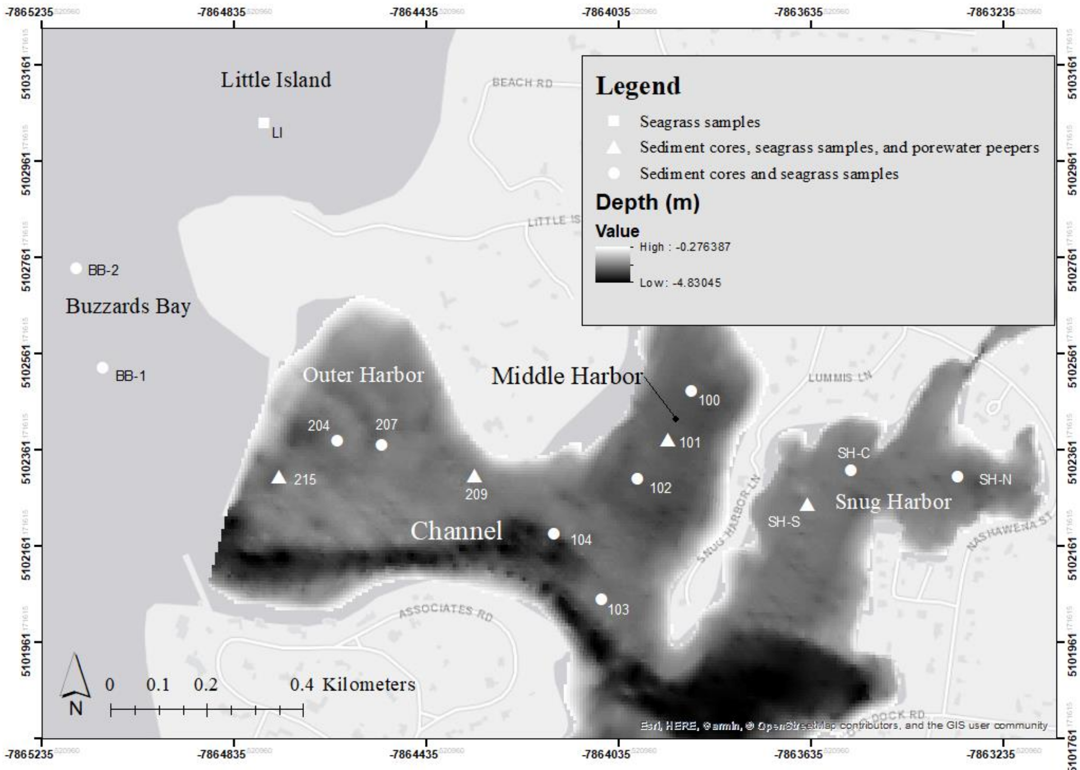

Fig. 3: major sampling sites and basins in West Falmouth Harbor. Figure created using ArcMap 10.6. Seagrass bed extent determined using side-scan sonar. Note that seagrass samples were not taken in Snug Harbor due to seagrass absence, but sediment cores were collected. Depth presented in meters at mean higher high water, ranging from 0.3 – 4.8 m. Triangles indicate porewater peeper locations.

Sediment analyses

In July 2017, I extracted 6.4 cm diameter (acrylic coring tubes) sediment cores to ~12 cm sediment depth at 3 sites in Snug Harbor, 2 sites in the Middle Harbor, 2 sites in the Outer Harbor, and 3 sites in between the Middle and Outer Harbors near the channel region (n=10, Fig. 3). Each of these sites in WFH has been assessed for seagrass aboveground biomass and seagrass tissue isotopic composition during surveys almost annually by members of the Howarth-Marino Lab since 2005. In July 2018, I sampled cores at every site listed above and in August 2018, added 3 new sites without historic seagrass survey data to assess seagrass health parameters just outside of WFH, and in regions where seagrass has expanded in the northern Middle Harbor since 2005 (Fig. 3). I separated cores into 2-cm sections, dried the sections at 100

°C for 48+ hours, and ground the samples using a mortar and pestle. I then measured total sulfur, total carbon, and organic matter in each core section. I analyzed total sulfur by direct combustion and infrared detection using a LECO S632 Sulfur Determinator in 2017. In 2018, I rinsed sediments with deionized water to eliminate salts and prevent corrosion of the column, and then analyzed for total solid-phase C and S (see below) using an Elementar Vario CNS element analyzer with 10 mg dried sediment samples in aluminum foil balls. After rinsing sediments to remove dissolved forms of S, measured sulfur can be termed total solid-phase sulfur, which I will refer to throughout the rest of the paper. I measured organic matter content via loss on ignition in both years (Heiri et al., 2001).

Porewater analyses

To measure porewater concentrations of total soluble sulfide, sulfate, and chloride, I deployed four porewater "peepers" (Teasdale et al., 1995) in July 2018, one in each basin (Outer Harbor, Middle Harbor, Snug Harbor) and one in the channel (Fig. 3). I deployed all porewater peepers in vegetated locations, except in Snug Harbor where seagrass is absent. Porewater peepers were deployed once before in WFH in 2007, prior to the Snug Harbor seagrass die-off, with one peeper in a vegetated portion of Snug Harbor, and a second in a nearby unvegetated location. The peepers, which are ~36 cm in length, are PVC wedges each with 14 wells that are placed below the sediment surface, and covered with a semi-permeable amphoteric, nylon membrane with 0.2 μm pores. I filled each well with distilled, deoxygenated water in a 100% N_2-atmosphere glove bag. We deployed the peepers below the sediment surface for two weeks. While placing the peepers in the sediment, I noted which of the 14 wells was the first below the sediment-water interface. The peepers provide a 2-week average of porewater ionic concentrations at each site. During collection, I and other divers, equipped with SCUBA gear, used N_2 atmosphere bags held upside-down in the water column. We slid the peeper directly from the sediment into the N_2 bag, immediately sealed the bag, and flushed the bags with N_2 from a small gas cylinder on the boat. Peepers stayed in anoxic conditions before and during deployment, as well as during porewater sample extraction in the lab, described below.

To assess total soluble sulfides, I used the methylene blue method of Gilboa-Garber (1971) as adapted by Howarth et al. (1983). To standardize the analysis, I first created a stock solution of 3.5 mM Na_2S by breaking off a small piece of Na_2S crystal, which I rinsed with DI and patted dry with a Kimwipe, then quickly weighed. I immediately dissolved the Na_2S crystal in N_2-deoxygenated deionized water in a sealed volumetric flask. I used deoxygenated deionized water to dilute the stock solution to 7 known concentrations of sulfide between 350 μM – 3.5 mM. Working in the glove-bag, I used a syringe to pierce the membrane and extract porewater samples from each well. I then introduced the samples to a 2% zinc acetate solution, resulting in the precipitation of the sulfide as ZnS to prevent further chemical oxidation of sulfide. We placed the samples in the dark in a refrigerator for approximately 2 hours, and then added to each sample a reagent solution composed of HCl, $FeCl_3$, and n,n-dimethyl-p-phenylene diamine. Sample solutions sat in the dark for 3 hours before spectrophotometric analysis at an absorbance of 670 nm.

Prior to storing sulfate and chloride samples for analysis, I bubbled the samples with N_2 gas fitted with a water trap to remove soluble sulfides. Samples were kept refrigerated and in darkness for 2-3 months prior to analysis. I assayed sulfate and chloride concentrations after a 250:1 dilution, un-filtered, using a ThermoScientific ion chromatographer (Haddad & Jackson, 1990).

Seagrass analyses

From 2005-2018, members of the Howarth-Marino lab group, our colleagues at the Marine Biological Laboratory (MBL), and the University of Virginia (UVA) collected grasses in WFH every year in July from 24 sites, including 10 sites from Snug Harbor prior to the 2010 die-off. Data from 19 sites from these earlier surveys are included in my leaf-tissue sulfur isotopic composition analysis. Members of our lab group analyzed seagrass leaf tissue $\delta^{34}S$ almost annually beginning in 2005. They carefully scraped epiphytes off the youngest (blade 1), and second- and third-youngest (blade 2 + 3) blade from samples taken at each site (Table 1) and dried the leaf tissues at 60 °C for 48+ hours. They ground tissues in a mortar and pestle containing liquid nitrogen and re-dried before analysis. They pooled 3 to 10 plants from a single site and age class into one sample to achieve the necessary sample volume. They analyzed between 1- and 6- replicates of each pooled sample. The MBL Stable Isotope Lab at the Ecosystems Center performed the analysis of our leaf tissue samples for $\delta^{34}S$ composition.

Along with the help of lab-mates and other SCUBA divers, I collected seagrass samples from 16 sites during one sampling period in July 2017, and then analyzed for leaf area, and epiphyte (for the purpose of this study, I refer to all epibiota on the surface of the plant as epiphytes) biomass. I and other divers took *in-situ* measurements of seagrass density using a 0.25 m^2 PVC quadrat, with three density counts at each site. In 2018, I and colleagues carried out two sampling periods, in July and August.

In July of 2018, I selected 8 sites for seagrass sampling due to their distribution along a gradient that spanned the nitrogen-enriched Middle Harbor region—which is the second-most nutrient-impacted basin in WFH, behind the now-unvegetated Snug Harbor—to the less nutrient-impacted Outer Harbor (Fig. 3). In addition to the measurements made in 2017, I added near-infrared (NIR) photography and quantification of above-ground vs. below-ground biomass, described below. At each site I harvested 5-10 plant samples. In August 2018, we replicated the above methods, and determined plant chlorophyll content, described in detail below. In this August sampling period, I harvested from 5 replicate sites from earlier in the summer, as well as 4 new sites, 3 of which occurred just outside the bounds of West Falmouth Harbor (Fig. 3) to serve as comparisons (Table 1). I processed all plant samples within 48 hours of harvest and kept samples refrigerated and in darkness prior to analysis.

I scraped epiphytes off of plant surfaces and then took multispectral photographs of plants, including bands in the NIR, red, green, and blue wavelengths. The physical structure of live photosynthetic vegetation causes it to reflect highly in the NIR wavelengths (750-1100 nm), a process referred to as the chlorophyll effect (Mangold et al., 2013). *Z. marina* tissue reflects light most strongly between 750-900 nm (NIR) and absorbs red wavelengths between 610-700 nm (Barillé et al., 2010). I photographed plants using a dual-camera set-up: an Agrocam NDVI Pro NIR-G-B camera capturing ~800 nm wavelength for NIR, and a GitUp Git 2 Pro action camera equipped with an Agrocam RGB lens capturing ~630 nm red. Despite being different brands, the cameras contained the same internal image processor. The cameras were held in a plastic frame in a fixed position over an 8” by 11” by 26” white polystyrene box set up to exclude external light. A set of lights within the box emitted only in the NIR and red wavelengths. At the beginning and end of each day, I photographed a color-standardization card to ensure my image capture and lighting did not change throughout the study. I gently patted seagrass blades dry, placed the samples in the box, and photographed blades twice on one side with each camera. The NIR and red band set-up was used to establish a measure of normalized difference vegetation index (NDVI) for each plant (Myneni et al., 1995). I then used the ArcGIS Pro software suite to achieve a measure of NDVI, between -1 and +1, by measuring blade area using a mix of supervised and unsupervised pixel classification, followed by manual selection of blade area, where needed. I then created an NDVI raster through the equation: $(NIR_{800} – Red_{630}) / (NIR_{800} + Red_{630})$. I recorded, for each blade on each plant, an average pixel NDVI value, as well as a minimum and maximum pixel value, and a standard deviation using the ArcGIS Zonal Statistics tool.

To measure aboveground and belowground biomass, I harvested terminal shoots with at least 7 cm of horizontal rhizome attached (Short et al. 2006). In the lab, I trimmed the rhizomes of all samples to include 7 cm horizontal rhizome, and only shoots attached to that 7 cm were included in the above-ground biomass count. Plants with large portions of aboveground biomass missing due to leaf breakage were excluded from analysis, except in cases where this was characteristic of nearly all plants at a site, which was the case in the northern Middle Harbor (sites 100 and 101). For these sites, we included only plants with the least amount of breakage possible. Following rhizome separation, I scraped blades and meristems to remove epiphytes and dried the aboveground tissue samples in a drying oven at 60 °C for 72 hours. I carefully cleaned roots and rhizomes of sediment and detritus in a distilled water bath and dried samples at 60 °C for 72 hours. I then measured the dry weights. Additionally, I collected epiphytes from the blades by scraping with a razor blade, dried them at 60 °C for 72 hours, and then weighed them. We then placed the epiphyte samples in a muffle furnace at 500 °C for 4 hours and measured the difference between weights to assess the amount of organic epiphyte biomass.

I kept seagrass samples frozen at -80 °C for 1 month prior to analysis of a subset of samples for chlorophyll content using the method described by Dennison (1990). I soaked 5 cm^2 of seagrass blade for 10 minutes in 3 ml 100% acetone at room temperature under room light, ground samples in an ice bath in a dark hood using a mortar and pestle, and combined with 10 ml

80% acetone, 20% DI water solution per cm^2 blade area. I used only fully-intact blades to assess chlorophyll content. I chose to use the top 5 cm^2 of each blade for analysis, as I determined from a subset of tests on individual segments along a blade that the blade tip represented a near-average value for the rest of the blade. Samples sat in a dark, chilled cooler for two hours, prior to analysis of sample concentration on a spectrophotometer at 663 nm, 645 nm, and 720 nm. I estimated chlorophyll a and b after Arnon (1949).

Results

Sediment and porewater data

Total carbon values measured in 2018 spanned 2 to 7% (mean: 5.1%) in the Middle Harbor; Outer Harbor carbon occurred in the range of 1 to 5% (mean: 1.9%); and Snug Harbor values spanned 1 to 4% (mean: 3.6%). Carbon values were significantly different between all sub-basins (Snug vs. Middle: $p = 0.006$; Snug vs. Outer: $p = 0.0007$; Middle vs. Outer: $p = 4.3 * 10^{-5}$, single-factor ANOVA). Total carbon at all sites showed no consistent trend with sediment depth (Table 2).

As with total carbon, organic matter was highest in the Middle Harbor (5 to 16% organic matter by mass; mean: 12%), and Snug Harbor (5 to 10% organic matter; mean 8%), and lowest in the Outer Harbor (1 to 11% organic matter; mean: 6%) in both 2017 and 2018 (Fig. 4, Table 3). Seagrass sites outside WFH generally contained less than 1% organic matter. Organic matter was significantly greater in the Middle Harbor and Snug Harbor than the Outer Harbor ($p=0.012$, single-factor ANOVA), but the apparently higher concentrations in the Middle Harbor compared to Snug were not substantially different ($p = 0.21$, single-factor ANOVA). Sites outside WFH showed significantly lower organic matter than those within any of the basins of

WFH (p=0.002, single-factor ANOVA). Organic matter at all sites generally declined with sediment depth (Fig. 4, Table 2).

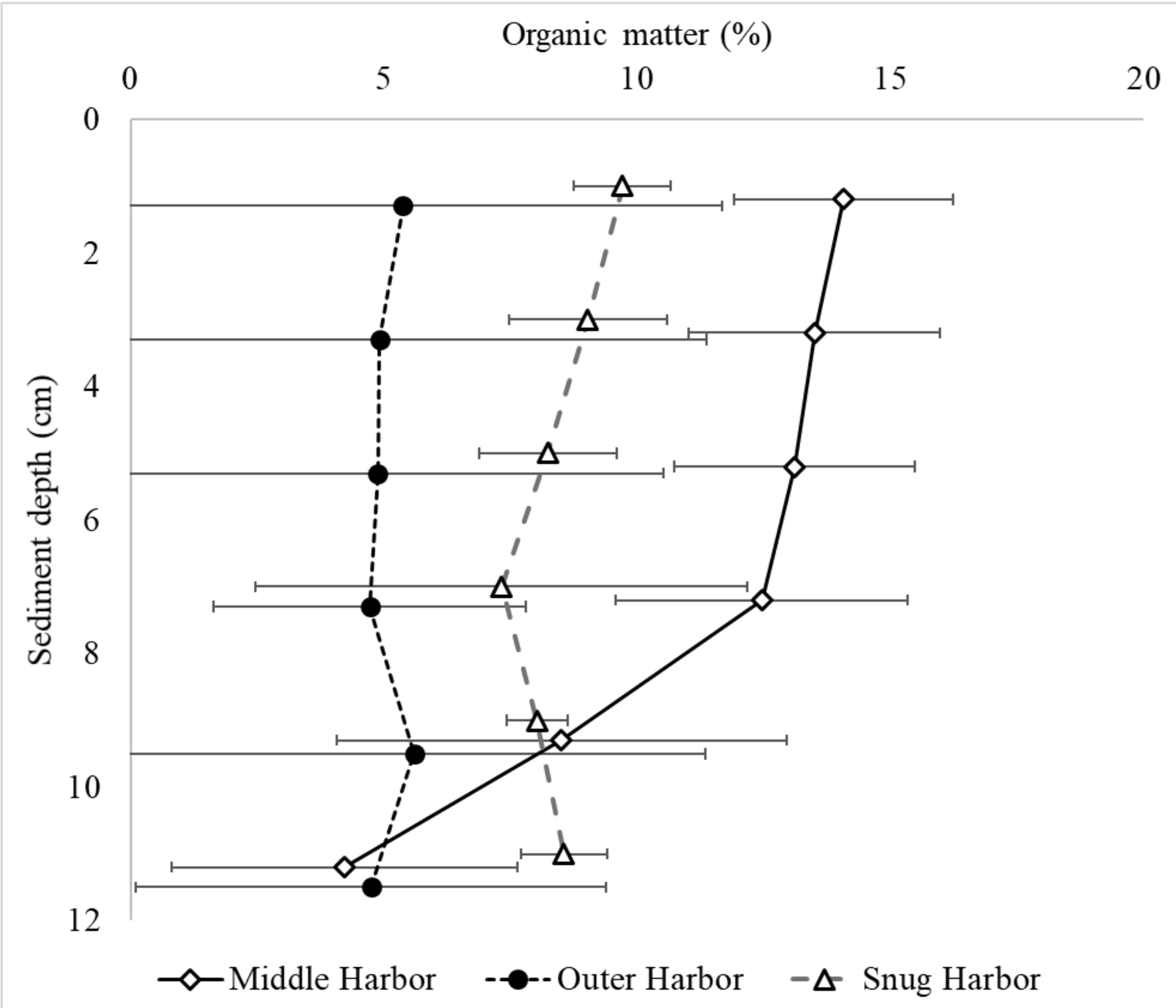

Fig. 4: LOI-derived organic matter for multiple sites in West Falmouth Harbor. Data from July 2018. Each bar represents the average measurement of cores in the specified basin. Error bars represent 95% confidence interval.

LOI-derived organic matter values were tightly correlated with total carbon values collected using CNS analysis, with a regression R^2 of 0.97 (Fig. 5). Carbon fit organic matter with a slope of 0.42 when assigned a 0 intercept, suggesting that across WFH, carbon makes up about 42% of organic matter on average. However, there are notable deviations from that average, particularly in sandy, low-organic matter regions of the Outer Harbor and outside of West Falmouth Harbor. Carbon to organic matter ratios at Outer Harbor sites 207 and 209 were on average 36%, much lower than most other sites in WFH, and likely indicative of methodological issues. I suspect that rinsing the sediments to remove salt also removed some portion of dissolved organic carbon from the sample, which would have artificially lowered the carbon to organic matter ratio. This would have led to greater deviations in the low-organic sediments, as we see in the data, because the loss of a small amount of DOC from the sample would represent a greater proportion of loss where organic matter is smaller. Additionally, we may see deviations due to differential carbonate content between samples.

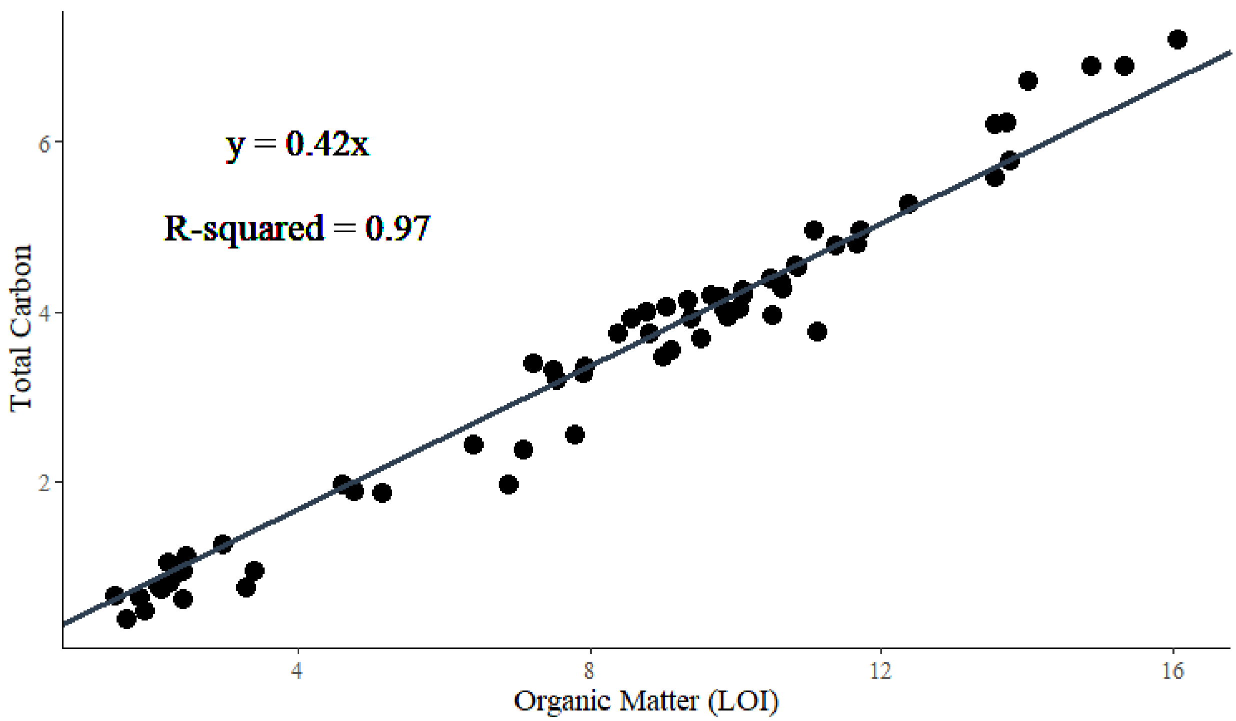

Figure 5: LOI-derived organic matter versus total carbon in West Falmouth Harbor, plotted through a forced-0 intercept.

The molar ratio of sulfate to chloride (SO_4^{2-}:Cl^-) in the porewater decreased with depth at all sites and was lowest in the Middle Harbor. Globally, ocean water SO_4^{2-}:Cl^- molar ratio remains constant at 0.513; in sediments, chloride is conserved while sulfate is reduced, leading to lower sediment sulfate to chloride ratios. Factors contributing to lower SO_4^{2-}:Cl^- ratios include the rates of reduction of sulfate to sulfide (Hines et al., 1989), bioturbation, sulfide reoxidation, and exchange of porewaters with the water column (Marvin-DiPasquale et al., 2003). Bottom water from our site near the sediment-water interface had a 0.05 SO_4^{2-}:Cl^- molar ratio (Fig. 6). In surface sediments near the Middle Harbor, SO_4^{2-}:Cl^- was 0.05, and decreased to 0.02 by 14 cm depth. Site 209 on the eastern side of the Outer Harbor spanned from 0.05 at the surface to 0.04 at depth. Site 215 in the western Outer Harbor spanned 0.05 to 0.04. In Snug Harbor, SO_4^{2-}:Cl^- spanned 0.05 to 0.03. The Middle Harbor had significantly lower SO_4^{2-}:Cl^- than the Outer Harbor sites and Snug Harbor (Middle and Outer Harbor, $p = 1.6 * 10^{-5}$; Middle and Snug Harbor, $p = 0.006$, single factor ANOVA).

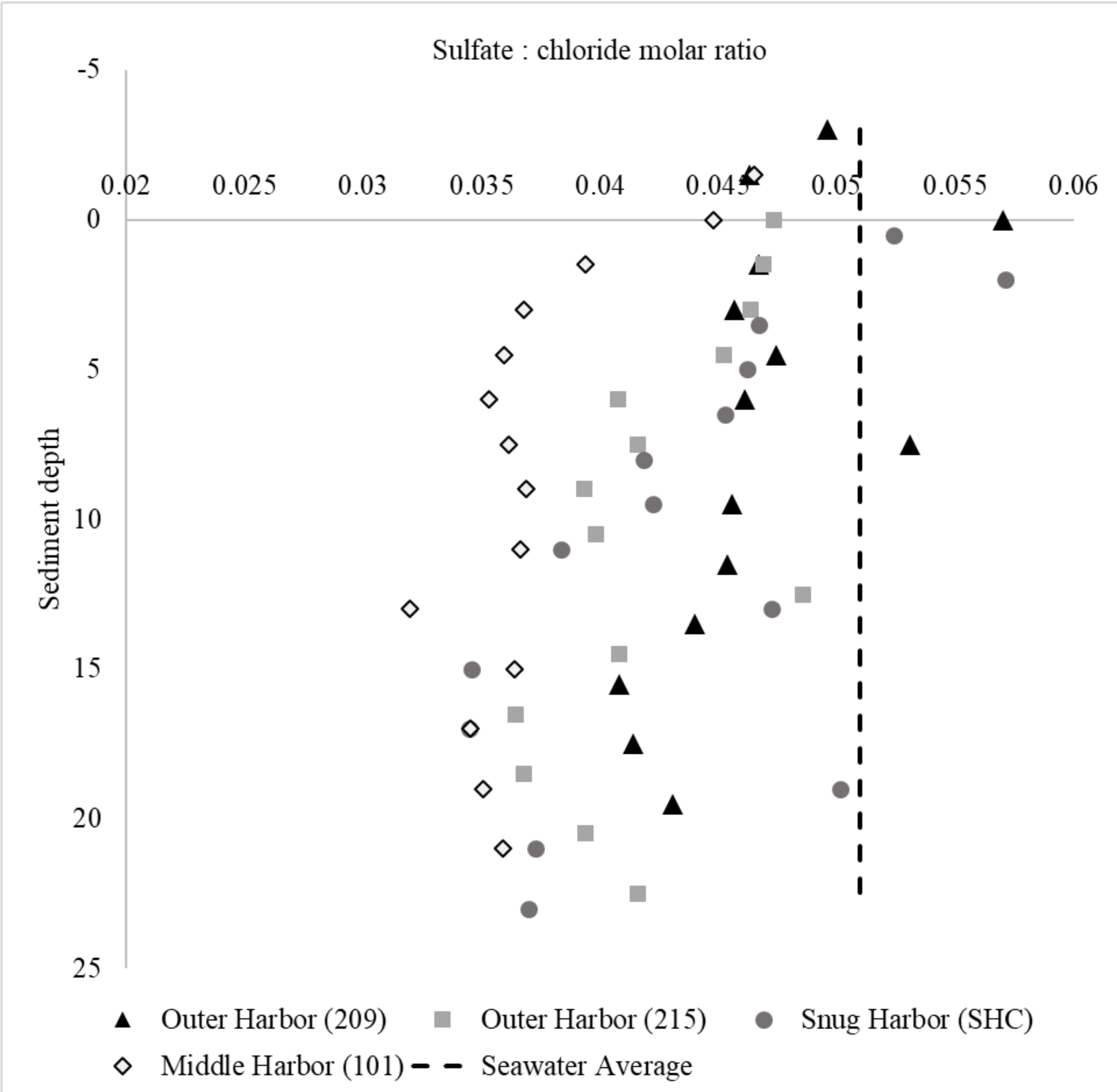

Figure 6: Sulfate : chloride molar ratios in West Falmouth Harbor with depth. Depth 0 represents the sediment-water interface. Dotted line represents the average seawater sulfate : chloride molar ratio.

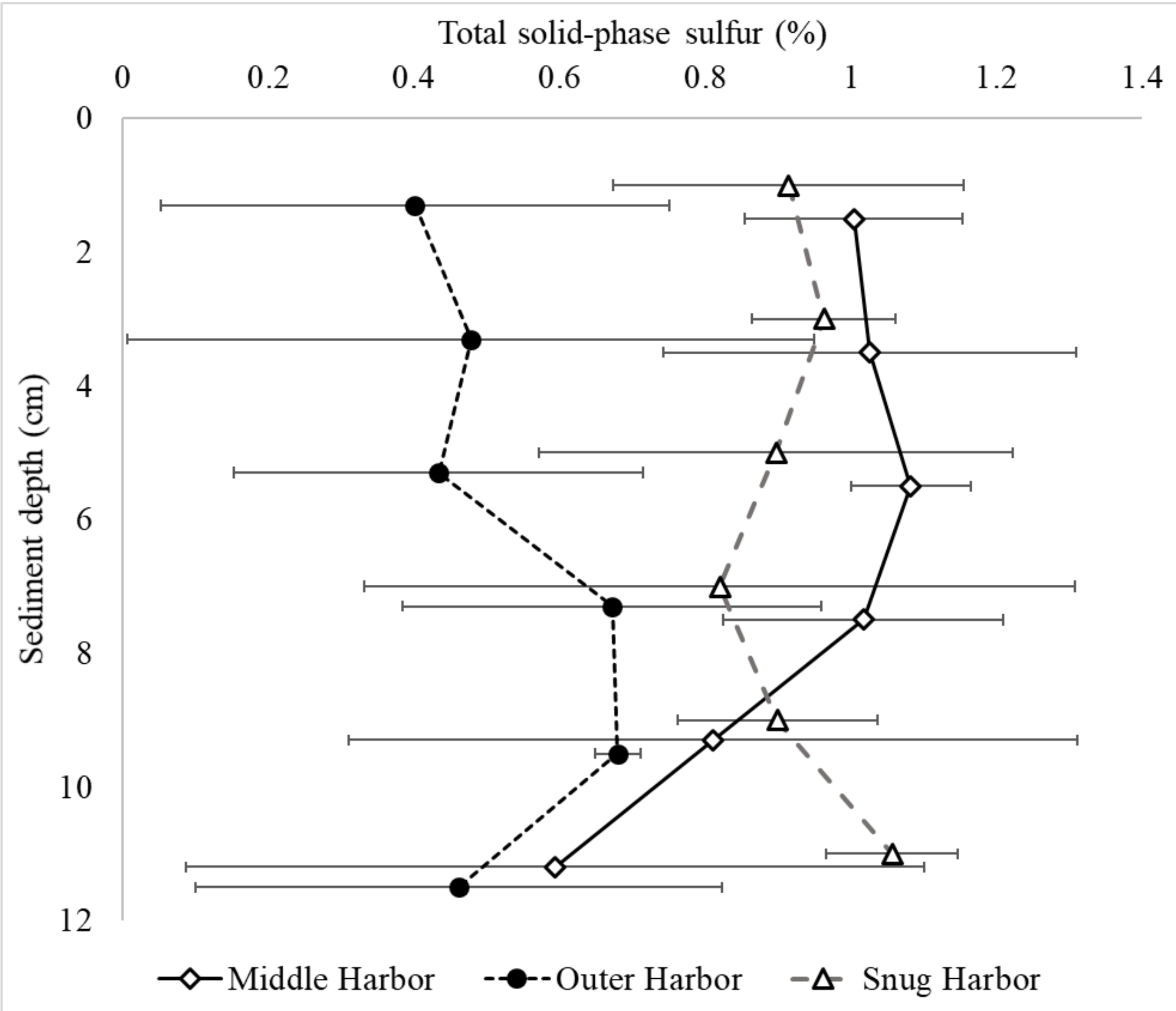

Fig. 7: Total solid-phase sulfur across three basins in West Falmouth Harbor. Significant difference between Outer Harbor and other basins. Data from July 2018. Each bar represents an average of 3 cores from the specified basin at the given depth class. Error bars represent 95% confidence interval.

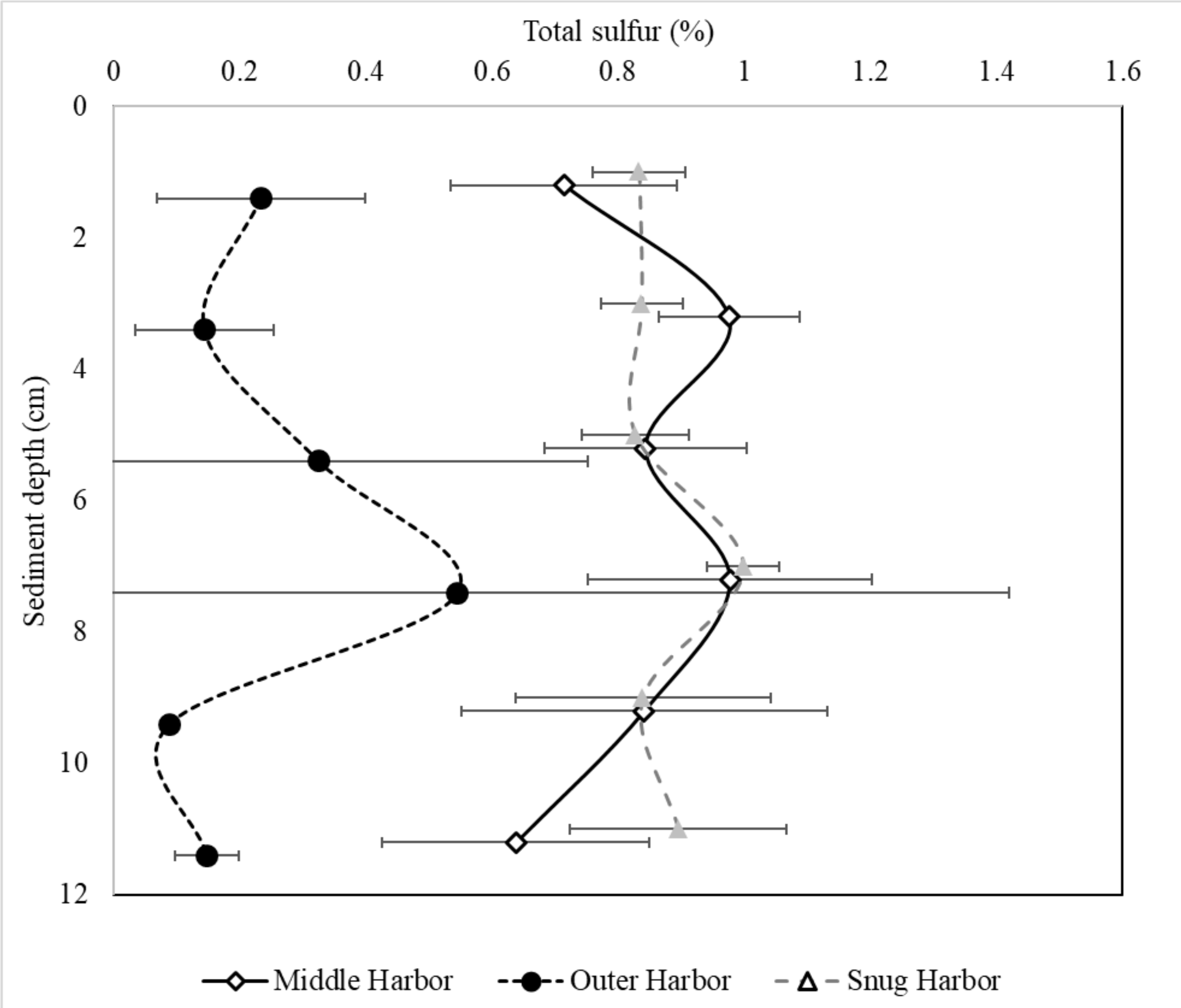

Fig. 8: Total sulfur across three basins in West Falmouth Harbor. Significant difference between Outer Harbor and other basins. Data from July 2017. Each bar represents an average of 3 cores from the specified basin at the given depth class. Error bars represent 95% confidence interval.

Total solid-phase sulfur, the portion of sulfur remaining after rinsing dissolved forms of sulfur from sediment, was greater in Snug and Middle Harbor than in the Outer Harbor (Table 3) in 2018 (Fig. 7), and total sulfur in 2017 followed the same pattern (Fig. 8). The Outer Harbor had significantly lower levels of total sulfur ($p=5.7 * 10^{-19}$, single-factor ANOVA) and total solid-phase sulfur ($p=3.6 * 10^{-10}$, single-factor ANOVA) than the other two basins. Snug Harbor and the Middle Harbor were not statistically different in total sulfur or total solid-phase sulfur. Total sulfur and total solid-phase sulfur showed no consistent change with depth throughout the length of the cores (Table 2). At sites where both porewater peepers and sediment cores were extracted, we can combine total sulfur with porewater sulfide and sulfate to get a measure of total sulfur in 2018. This value is on average 50 % higher than the 2017 data. At site 207, average total sulfur in 2018 was 0.52 %, compared to 0.11 % in 2017. Site SH-2 had a 2018 average of 1.1 %, and a 2017 average of 0.89 % total sulfur. Site 101 had 1.2 % in 2018, and 0.78 % in 2017. It is unlikely that this is a true temporal trend, and probably instead reflects methodological differences between the two combustion analyzers used.

Total soluble sulfides were strikingly higher in the Middle Harbor than at other sites, with values between 2.0 and 2.7 mM (Fig. 9). Concentrations in the other basins were far lower, 350 μM to 1 mM in OH and 200 to 700 μM in Snug Harbor. In the deep, sandy channel region of WFH between the Middle and Outer Harbors (Fig. 3), soluble sulfide concentrations were lower at only 30 and 100 μM sulfide. The difference between Middle Harbor and the other basins was highly significant according to a single-factor ANOVA test ($p = 1.6 * 10^{-14}$). Sulfide increased with depth in all porewater profiles except for the Middle Harbor, where it remained uniformly high across depth (Fig. 9). Notably, the porewater sulfide concentrations in the vegetated portions of Snug Harbor measured by colleagues at MBL in 2007 (Giblin et al., unpublished data), three years before the die-off, were similar to the current values seen in the Middle Harbor (Fig. 10). However, in the 8 years since the loss of seagrass in Snug Harbor, porewater sulfide values in Snug Harbor have decreased to levels comparable with the Outer Harbor.

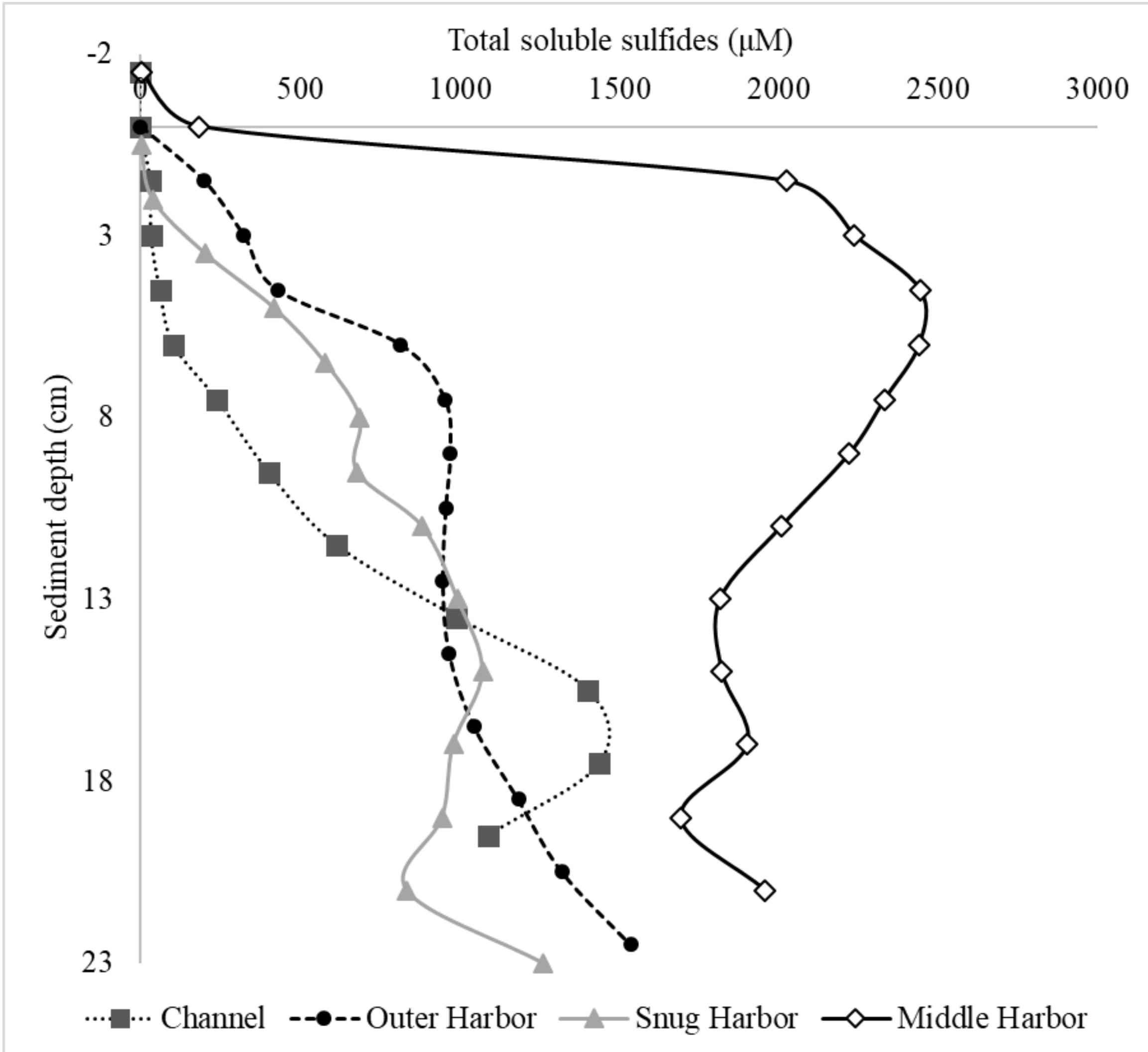

Fig. 9: Porewater total soluble sulfide concentrations at four sites across West Falmouth Harbor. Note the very high sulfide concentrations and deviation from depth-sulfide pattern in the Middle Harbor. Each dot represents one peeper well measurement. Data from July 2018.

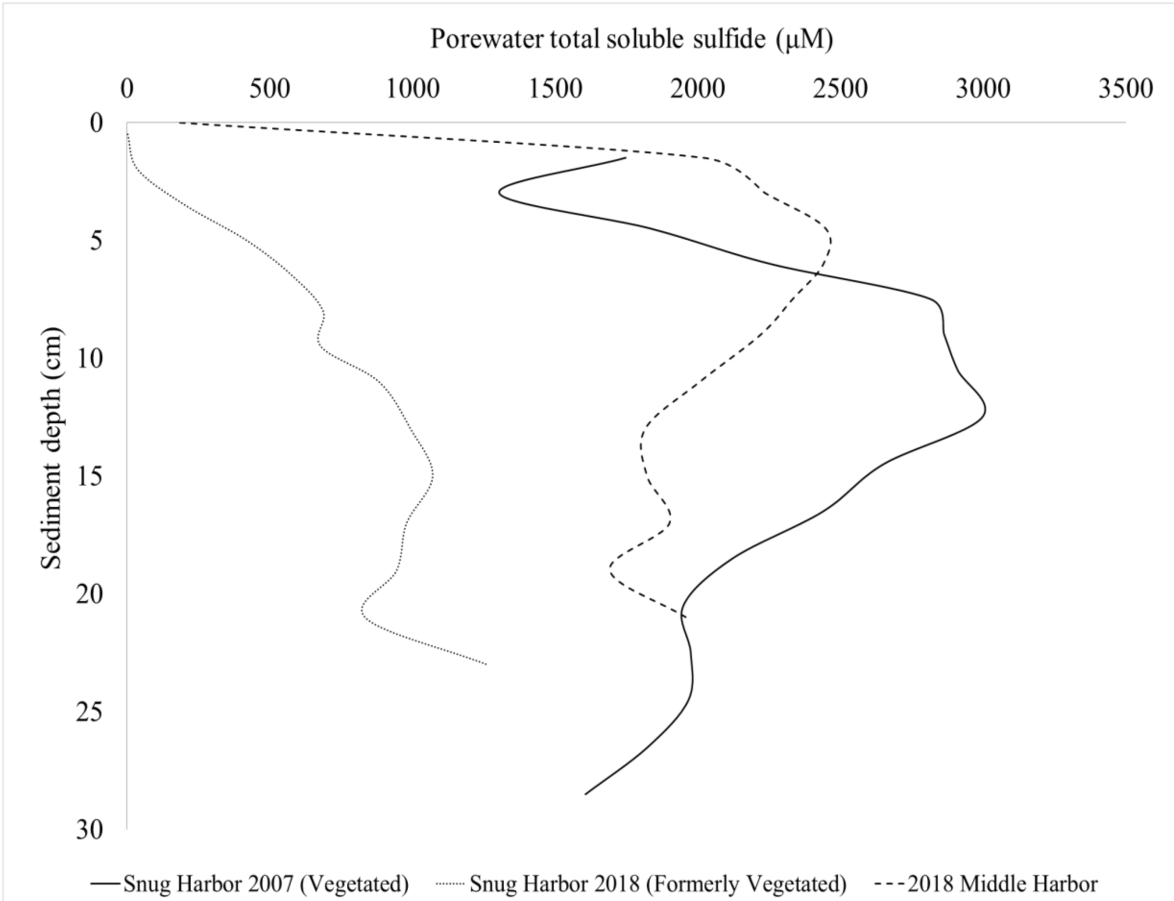

Fig. 10: Sediment sulfide concentrations in West Falmouth Harbor's Snug Harbor basin through time compared with the Middle Harbor. The dashed line represents current sulfide concentrations in the Middle Harbor basin, which are similar to values in Snug Harbor prior to a 2010 seagrass die-off event.

Seagrass data

The Middle Harbor aboveground biomass during July 2018 was 350 to 650 g dry weight (DW) per m^2 (Fig. 11). Outer Harbor aboveground biomass spanned 200 to 400 g DW per m^2. Below-ground biomass was highest (80 to 130 g DW per m^2) in the Outer Harbor, and lower in the Middle Harbor (50-80 g DW per m^2). I found a significant difference between the Outer and Middle Harbors for below-ground biomass ($p = 0.0001$, single-factor ANOVA), but not for aboveground biomass ($p=0.60$, single-factor ANOVA).

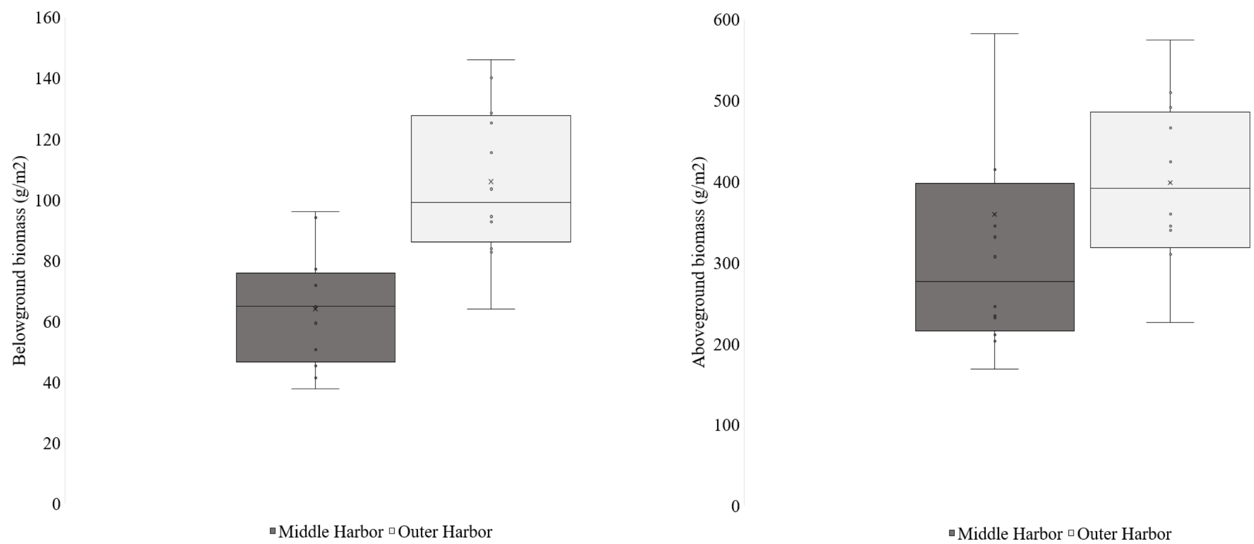

Figure 11: Belowground and aboveground seagrass biomass in West Falmouth Harbor, July 2018. Boxplots span the full range of data collected. The Middle Harbor is an average of 4 sites (101, 102, 103, 104), and the Outer Harbor is an average of 4 sites (204, 209, 207, 215). Boxplot whiskers cover the full scope of measurements (except where dots off the line signify outliers), bar represents mean, box contains 1st and 3rd quartile of data. Note the different scales for belowground and aboveground biomass.

Total epiphyte biomass in the Middle Harbor in July 2018 ranged between 0.2 to 0.5 mg epiphyte per cm^2 leaf surface area. In the Outer Harbor, epiphyte biomass ranged from 0.05 to 0.5 mg epiphyte per cm^2 leaf area (Table 4). When assessed in mass per mass units, epiphyte biomass in the Middle Harbor ranged between 30 to 130 mg epiphytes per g seagrass (DW), and 50 to 110 mg per g in the Outer Harbor. Epiphyte biomass was not significantly different by basin ($p=0.36$, single-factor ANOVA). Organic epiphyte biomass in the Middle Harbor ranged between 0.1 to 0.3 mg epiphyte per cm^2 leaf surface area (20 to 100 mg organic epiphyte per g seagrass). In the Outer Harbor, organic epiphyte biomass ranged from 0.05 to 0.3 mg epiphyte per cm^2 leaf area (30 to 75 mg organic epiphyte per g seagrass). Organic epiphyte biomass was not statistically different by basin ($p=0.18$, single-factor ANOVA), but showed a slight trend toward greater values in the Middle Harbor than total epiphyte biomass.

Leaf tissue $\delta^{34}S$ was significantly lighter in Snug Harbor prior to the mortality event in 2010, relative to the Outer Harbor (Fig. 12). $\delta^{34}S$ values in the Middle Harbor between 2013 and 2017 were in the same range as Snug Harbor $\delta^{34}S$ values prior to 2013, between +5 and -5 ‰, clustered around 0 ‰. While the data are variable, the Outer Harbor has trended toward isotopically lighter $\delta^{34}S$ values in 2015-2017 (Fig. 13). In 2017, the average value for the Middle Harbor was 0.0 ‰, and the Outer Harbor was -0.1 ‰. This is a stark decline from the average from 2013-2016, which was +8.0 ‰ in the Outer Harbor and +1.6 ‰ in the Middle Harbor. Prior to the seagrass die-off in Snug Harbor, leaf tissue $\delta^{34}S$ averaged 0.0 ‰ from 2005-2010. Leaf tissue $\delta^{34}S$ values in the last year of seagrass presence in Snug Harbor were as low as -7.5 ‰. In 2017 in the Outer Harbor, site 213 (not pictured in Fig. 3., slightly south of site 215) had an average value even lower than that, at -9.1 ‰.

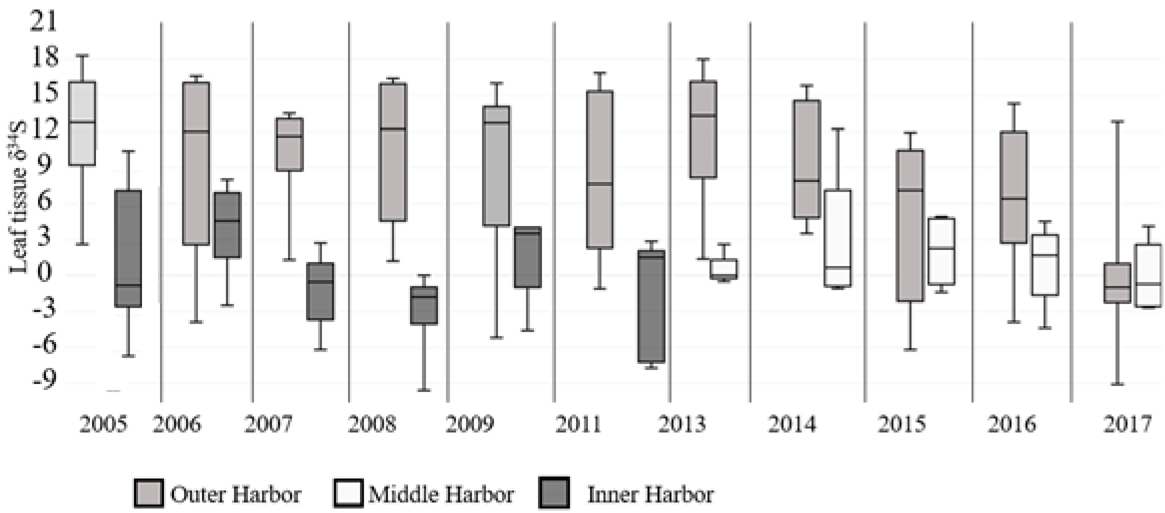

Fig. 12: Seagrass leaf tissue sulfur stable isotopic composition in West Falmouth Harbor through time. Boxplot whiskers cover the full scope of measurements, bar represents mean, box contains 1st and 3rd quartile of data.

NDVI values were generally clustered above zero, indicating greater NIR reflectance than red reflectance and the presence of photosynthetically active vegetation (Kiage & Walker, 2009), with August values higher overall than July. Blade averages ranged from -0.1 to +0.2 in July, and -0.0 to +0.2 in August. In general, values were highest in the Middle Harbor in both months, with site 102, located in the center of the Middle Harbor (Fig. 3), exhibiting the highest values seen in both months. In July 2018, seagrass in the Middle Harbor had an average NDVI ~55% greater than seagrass in the Outer Harbor ($p=3.7*10^{-6}$, single-factor ANOVA). In August 2018, the difference between the two basins shrunk to a 10% greater value in the Middle Harbor, but still showed a significant difference ($p=0.03$, single-factor ANOVA). All WFH sites (Outer and Middle Harbor) together showed significantly lower NDVI than sites outside WFH ($p=0.002$, single-factor ANOVA).

Middle Harbor plants had an average concentration of 14 µg seagrass chlorophyll a+b per cm^2 leaf area (Table 4). The Outer Harbor values were similar and not statistically different at 13 $\mu g/cm^2$ ($p=0.48$, single-factor ANOVA). Sites outside WFH (Buzzards Bay and Little Island) had seagrass chlorophyll content averaging 10 µg per cm^2 leaf area (Table 4). The apparently lower chlorophyll content in sites outside of WFH had a p-value of 0.08.

Discussion

Organic carbon in WFH is greater than commonly observed for seagrass meadows. A review of 3,561 observations of organic carbon in seagrass meadows around the world by Fourqurean et al. (2012) found an average value of 2.0 % sediment organic carbon, and a median value of 1.4 %. In the Middle Harbor basin of WFH, carbon values average 5.0 %, greater than ~90 % of measurements reviewed by Fourqurean et al. (2012). Snug Harbor organic carbon averages 3.6 %, slightly less than the Middle Harbor. Prior to the die-off, Snug Harbor's vegetated sediments had a higher carbon value, at 4.4 % carbon (Marino et al., unpublished data). Organic carbon in Snug Harbor was comparable to, or greater than, the Middle Harbor prior to the die-off event as a result of seagrass particle trapping and primary productivity. The lower carbon found in currently un-vegetated sediments compared to the vegetated Middle Harbor sediments supports my hypothesis that seagrass particle trapping and seagrass and epiphyte primary productivity in eutrophic conditions can lead to highly organic sediments. Seagrass organic matter-trapping may benefit plants in nutrient-scarce conditions, or where limitations in light favor decreased depth and low turbidity (de Boer, 2007). However, in nutrient-enriched conditions, this strategy may not be beneficial. My data indicate that higher sediment carbon accumulation in eutrophic seagrass meadows can lead to accumulations of sulfide, declines in seagrass health, and potential loss of meadow area in situations where seagrass are subject to multiple environmental stressors.

Total solid-phase sulfur in the sediments of WFH are greater than commonly seen in estuaries. While there are very few reported total solid-phase sulfur values for seagrass meadow sediments, un-vegetated estuaries in North America have had sediment solid-phase sulfur values between 0.04 and 0.22% within the top 20 cm (Grant & Bathman, 1987). Sediment total solid-phase sulfur in 2017 in WFH exceeded this, with minimum values of 0.46% at sites in the Outer Harbor, and maximum values of 1.1% in the Middle and Snug Harbors. Total sulfur in 2017 also exceeds these expectations, despite being lower than 2018 total solid-phase sulfur overall. 2018 total sulfur—calculated by adding total solid-phase sulfur, sulfate, and sulfide together— is on average 0.2-0.4% higher in absolute percentages than 2017 total sulfur. This is likely due to methodological differences and small-scale site heterogeneity.

Sulfate:Chloride molar ratios in WFH are lower than many reported estuarine pore water ratio values, particularly in the Middle Harbor where values strongly decrease in SO_4^{2-} relative to Cl- at 0.035 at 12 cm (Table 2), and as low as 0.032 at 13 cm (Fig. 6). Ku et al. (1999) observed values declining from 0.051 at the sediment surface to 0.048 at 25 cm depth on a shallow mudbank site occupied by a multi-species seagrass meadow. I found much lower ratios in West Falmouth Harbor than Ku et al. found at their site, even at shallow depths, particularly in the highly sulfidic porewaters of the Middle Harbor, which may indicate high rates of sulfate reduction and lower levels of reoxidation of reduced-S.

Although dwarfed by Middle Harbor sulfide concentrations at 2.3 mM, soluble sulfides at 10 cm depth in the Outer Harbor exceed 1.0 mM, well beyond the sulfide concentration of 0.6 mM where Goodman et al. (1995) and Höffle et al. (2011) saw declines in seagrass health. Seagrass (*Z. marina*) mortality is high when experimentally exposed to sulfide concentrations above 600 μM (Höffle et al., 2011), concentrations far lower than those we measured in the Middle Harbor. Dissolved soluble sulfides tend to be low where seagrass beds are found, with a median value of 50 uM total soluble sulfides in seagrass meadows across the globe reported in a review by Terrados et al. (1999). In that review, only 1 site out of 22 had greater than 300 uM soluble sulfide, which contrasts starkly with our average value in the Middle Harbor of 2.3 mM, more than 7-times higher. Additionally, sulfide values in the Middle Harbor did not increase with depth like they did at other sites (Fig. 9), and instead were at their highest between 1-5 cm, likely resulting in high levels of sulfide stress on the belowground tissues of plants in the Middle Harbor.

A notable result in this study is the similarity between porewater soluble sulfide concentrations in Snug Harbor in 2007, and the Middle Harbor in 2018 (Fig. 10). Both basins are N-enriched and contained seagrass beds at the time of sampling, and their porewater soluble sulfide concentrations were the two highest we measured, with soluble sulfides in the vegetated area of Snug Harbor in 2007 averaging 2.2 mM, and the Middle Harbor in 2018 averaging 2.3 mM. In 2018, Snug Harbor sulfide concentrations in a formerly-vegetated portion of the harbor fell far below the 2007 values when measured in 2018, averaging 0.7 mM (Fig. 10). This observed decrease in porewater sulfide from 2.2 to 0.7 over the course of a decade following seagrass loss demonstrates the role of seagrass in elevating organic matter concentrations and leading to porewater sulfide proliferation.

Across all sediment depth classes and sites, rooting zone organic matter and porewater sulfide had a strong log-linear relationship (Fig. 13). Total soluble sulfide concentration increases on a logarithmic scale as organic matter increases linearly. The four basins group in different locations along the regression line, with the three points at the bottom of the line from site 209, the six points in the middle from Snug Harbor and Outer Harbor's site 215, and the uppermost points from Middle Harbor's site 101. This relationship was highly significant (p=0.00043, t-test) and likely reflects the use of organic matter by sulfate reducers, as well as a potential change in sediment redox condition at high organic matter values.

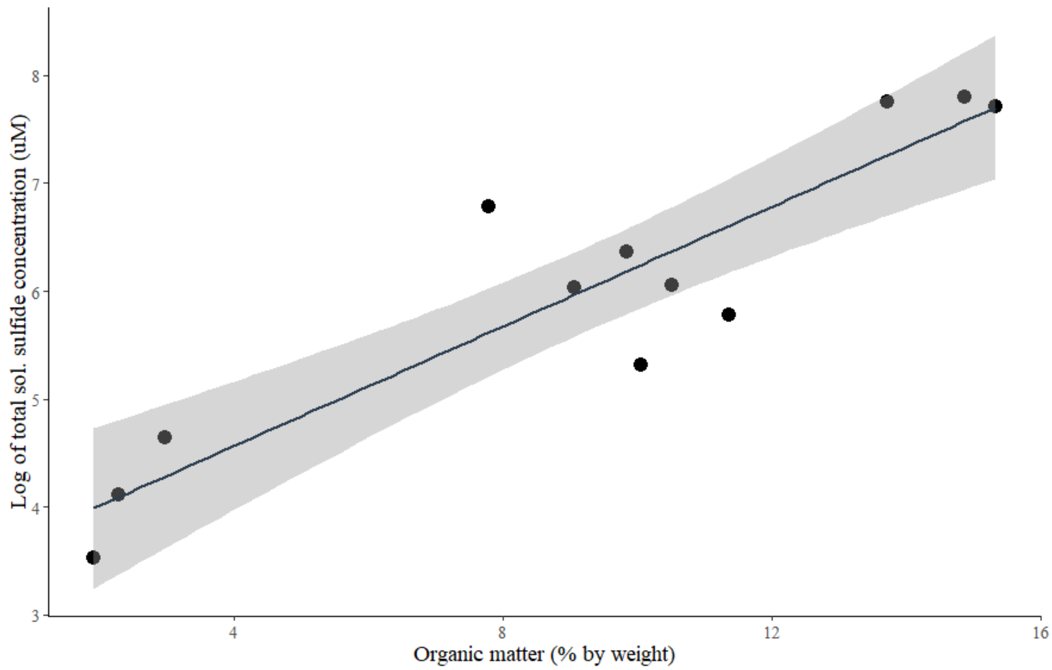

Fig. 13: Porewater sulfide concentration (µM) and percent organic matter across all peeper sites in West Falmouth Harbor, sediment depth 2-8 cm (n=12, p=0.00043, R2 = 0.833).   Gray bars represent 95% confidence interval. Note log scale.

Seagrass leaf tissue $\delta^{34}S$ in WFH is consistently higher in the Outer Harbor than the Middle or Snug Harbor, but the Outer Harbor has trended toward more depleted isotopic values in recent years (Fig. 13). From 2005-2010, the Outer Harbor's grasses had an average $\delta^{34}S$ of +10 ‰, compared to +0.0 ‰ in Snug Harbor. During this time period, individual plant $\delta^{34}S$ values in the Outer Harbor ranged from -5.2 to +18 ‰ (n = 41), while grasses in Snug Harbor ranged from -10 to +11 ‰. A sample of grasses taken from the Middle Harbor during this time period spanned -9 to +9 ‰. From 2011-2017, following the loss of vegetation in Snug Harbor, Outer Harbor values have declined from an average of +11.6 ‰ in 2013, to -0.1 ‰ in 2017. During that time, Middle Harbor values remained largely consistent, with averages between 0.0 and +3.0 ‰ each year. Individual site measurements in the Outer Harbor from 2013 to 2017 reached a maximum of +18 ‰ in 2016, and a minimum of -9 ‰ in 2017. Middle Harbor measurements ranged from -4.4 ‰ to +5 ‰ at four sites measured in the Middle Harbor, with a single sample much heavier (+12 ‰) at site 104 in 2014.

The $\delta^{34}S$ values in West Falmouth Harbor through time are more depleted than many previous studies. A review by Holmer & Hasler-Sheetal (2014) of 44 leaf tissue stable sulfur isotopic observations in *Z. Marina* found an average value of 4.0 ‰. Plants in the Middle Harbor had an average $\delta^{34}S$ value of 0.0 ‰ in 2017, with individual plant measurements as low as -2.7 ‰ (Table 3). One site in the western portion of the Outer Harbor, where there has been a considerable decline in leaf tissue $\delta^{34}S$ since 2014 (Fig. 13), had an average $\delta^{34}S$ value of -9.1 ‰ in 2017; this value is isotopically lighter than 98% of observations in the review by Holmer & Hasler-Sheetal (2014). Prior to Snug Harbor's mortality event, $\delta^{34}S$ leaf tissue values were as low as -7 to -10 ‰, with nearly 60% of measurements taken on Snug Harbor grasses between 2007-2010 found to be below 0 ‰. This finding implicates sulfide uptake and stress as a major contributor to the 2010 seagrass mortality event in Snug Harbor.

Nixon et al. (2001) noted that the mortality of seagrasses increases as the aboveground to belowground biomass ratio exceeds 4. Ratios higher than this are common within WFH, particularly in the Middle Harbor where the July average of sites 101, 102, 103, and 104 was 4.7 (Table 4). At sites in the more northern portion of the Middle Harbor (101 and 102), the average exceeded 6. The average above- to belowground biomass value in the Outer Harbor was 3.7. In August, values were lower at all sites due largely to a decrease in above-ground biomass, but the biomass ratio remained 25% higher in the Middle Harbor compared to the Outer Harbor. The August dataset contains additional sites from locations just outside of West Falmouth Harbor (Fig. 3). Average August values for the above- vs. belowground biomass ratio were 3.1 for the

Middle Harbor, and 2.9 for the Outer Harbor. For sites outside WFH, the ratio was 2.1, almost 40% lower than sites within WFH. 75% of sites in the Middle Harbor exceeded the 4.0 threshold reported by Nixon et al. (2001), with site 102 having 7-fold greater aboveground biomass than belowground biomass. In the Outer Harbor, only site 215, which also contains the highest levels of sediment organic matter seen in the Outer Harbor, had greater than 4 for its aboveground to belowground biomass ratio. The differences in this ratio in WFH were driven entirely by variations in belowground biomass, and not by aboveground biomass. In other systems, similar findings have indicated the effect of sediment conditions on seagrass resource allocation (Holmer & Nielsen, 1997).

Light availability, which we assessed through NDVI and chlorophyll content, appears to be influencing West Falmouth Harbor's seagrasses in addition to sediment condition. The NDVI and seagrass chlorophyll content relationship was linear and significant ($p=0.00003$, t-test), but contained large amounts of variance (Fig. 14). The level of variance matches that of previous work comparing vegetation indices to photosynthetic marine organisms, including macroalgae (Murphy et al., 2000). In the case of our data, I suspect that much of this variance is due to differences in epiphyte cover, as my data show a significant relationship ($p=0.02$, t-test) between total epiphyte cover and NDVI (Fig. 15). Even with epiphytes removed from seagrass blades prior to NDVI analysis, dark spots—likely necrotic lesions—were visible on NIR-band photographs of seagrass blades where epiphytes used to be.

Seagrass in the Middle Harbor appear to be light-limited based on their higher concentration of photosynthetic pigments when compared to Outer Harbor plants or plants outside WFH, as assessed through NDVI (Ralph et al., 2007). NDVI was ~25-50% greater on average in the Middle Harbor than the Outer Harbor (Table 4). Light stress in West Falmouth

Harbor probably occurs as a result of epiphyte biomass levels exceeding 0.5 mg DW epiphyte per cm^2 DW seagrass, as seen in plants in both the Middle and Outer Harbors. This conclusion is supported by the significant relationship between epiphyte cover and average blade NDVI (Fig. 15). Despite similar epiphyte densities across WFH, epiphytes are likely more of a problem in the Middle Harbor, as the light extinction coefficient in the Outer Harbor has found to be lower than other areas of WFH (del Barrio et al., 2014). Values at the high end of the range of epiphyte cover measured in July 2017 and 2018, at 0.5 mg epiphyte per cm^2 seagrass, can indicate 25 % light attenuation in temperate seagrasses and may be associated with seagrass meadow area reduction (Nelson, 2017). Epiphyte abundance estimates from previous years show a marked increase in epiphyte cover in WFH later in the growth season, with values exceeding 5 mg per cm^2 reported in September of 2018. The top 15 cm of the blade was consistently the most heavily colonized by epiphytes, with a median of 1.8 mg per cm^2 in August 2018 (n = 44, Marino et al., unpublished data). Our epiphyte biomass numbers are likely underestimates of true levels, as some epiphytes are loosely bound to seagrass surfaces and may be lost during sampling.

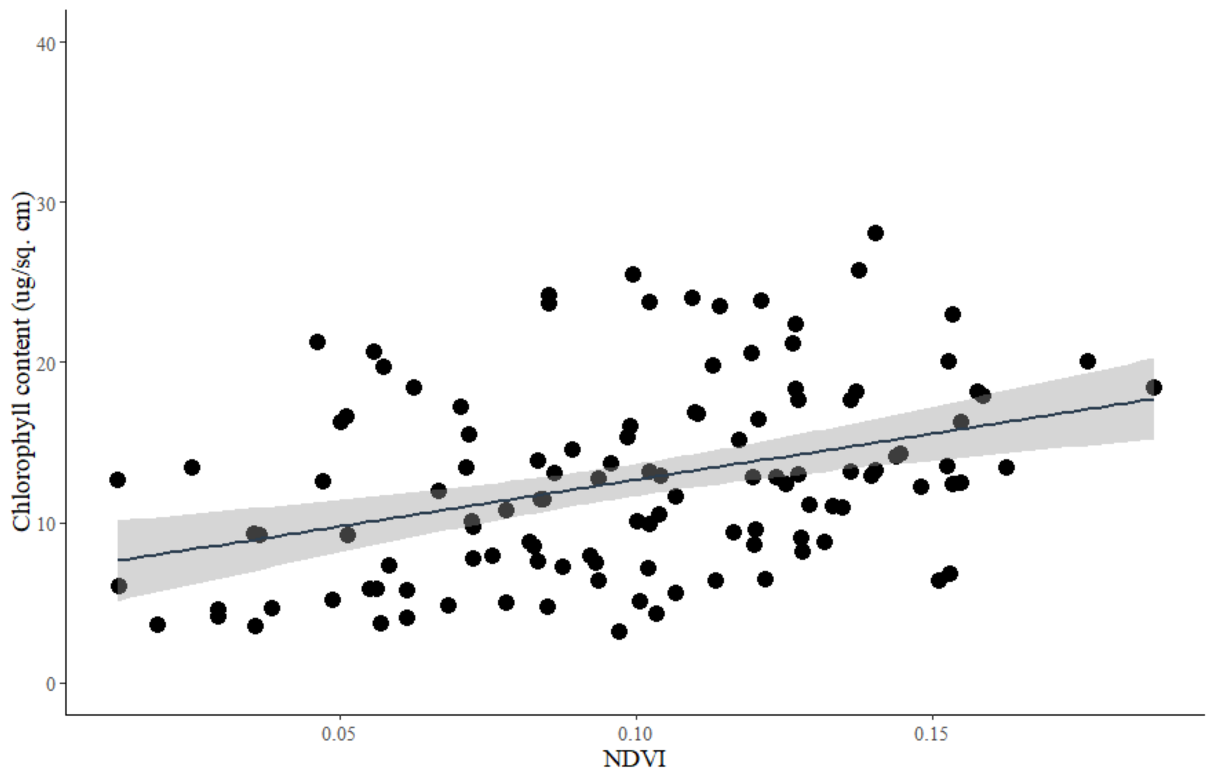

Fig 14: Chlorophyll vs. NDVI in seagrass samples analyzed in the lab. Each point represents one blade's average NDVI value vs. tip chlorophyll content. Despite noise, relationship is statistically significant: p=0.000031, n = 119. R^2 = 0.145. Gray bars represent 95% confidence interval. Samples harvested from West Falmouth Harbor and external sites, August 2018.

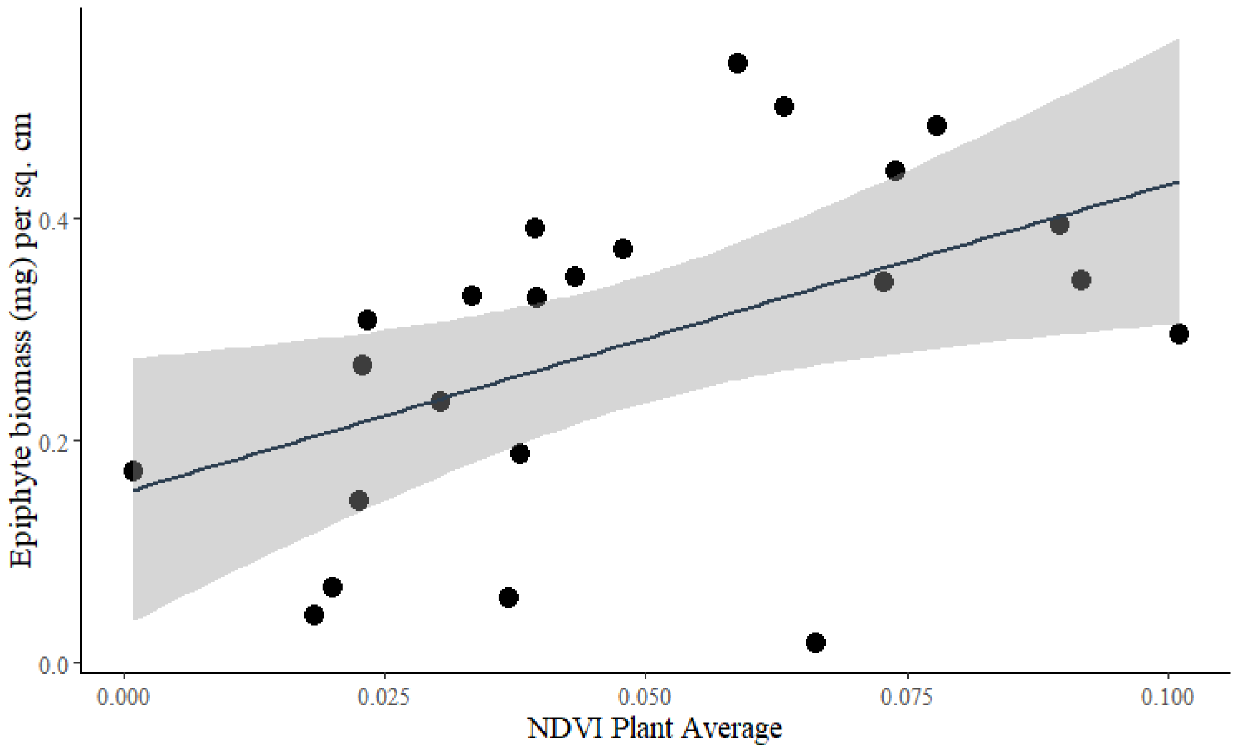

Fig. 15: Total epiphyte biomass per leaf area (plant average) vs. NDVI for July 2018 seagrass samples. Each point represents one plant (n = 23). NDVI increases linearly with increased epiphyte biomass ($p = 0.015$, $R^2 = 0.25$). Gray bars represent 95% confidence interval. NDVI represents whole plant average, and epiphyte biomass represents total epiphytes per cm^2 plant leaf area.

Goodman et al. (1995) demonstrated that in low-light conditions seagrass is more susceptible to sulfide toxicity, with symptoms of toxicity such as decreased photosynthetic rate commencing around 600-1,000 µM porewater sulfide. In meadows with higher exposure to light, seagrass show greater resistance, and can withstand greater concentrations of porewater sulfide (Goodman et al., 1995; Lamers et al., 2014). Attenuation of more than 25% of light reaching seagrass tissue due to epiphyte cover presents a serious loss of photosynthetic potential, reducing the capacity for the Middle Harbor's seagrass to resist sulfide toxicity using photosynthetically derived O_2. Despite total soluble sulfides as high as 3.0 mM found in Snug Harbor in 2007, seagrass in this region persisted until 2010. This may be explained by the impact of long-term exposure to sulfide concentrations above 2.0 mM on plant health

(Frederiksen et al., 2008). Seagrass may internally detoxify sulfide, using photosynthetically derived O_2 to oxidize S^{2-} to $10S^0$, but this may present a major drain on plant energy, and can break down in situations where multiple environmental stressors such as low light or CO_2 limitation reduce seagrass photosynthesis (Hasler-Sheetal & Holmer, 2015). High ratios of aboveground to belowground biomass like I observe in seagrasses in WFH's Middle Harbor basin may lead to further energetic expense on seagrass, as plants with relatively little rooting mass will need to rely on nutrient uptake from the water column, where nutrients are less concentrated and more variable, rather than the relatively concentrated, stable nutrient pool in the sediments (Hemminga, 1998).

A notable feature of West Falmouth Harbor's Middle and Snug Harbors is that they are low-energy, shallow (generally less than 2 m depth) basins protected from high wave-action. Seagrass here are capable of growing root and rhizome structures in the water column, rather than the sediment. I observed this behavior in the Middle Harbor summer 2018, and my colleagues collected photographic evidence of the phenomenon in 2017 (Fig. 16). This was observed several times before 2018 in WFH by members of the Howarth-Marino Lab, particularly in Snug Harbor in the years leading up to seagrass loss. Despite protecting roots and rhizomes from toxic sediments, this strategy may leave the below-ground tissue vulnerable to herbivory and could also lead to plants washing away during strong tides or storm-induced wave action. Further research is needed to understand this phenomenon, its causes, and its consequences.

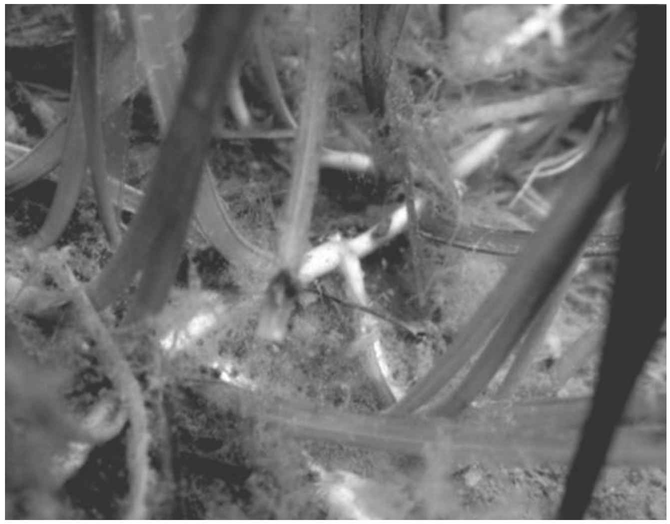

Fig. 16: Seagrass rhizome (white) sitting above sediment-water interface in WFH's Middle Harbor basin. Photographed July 2017 by Melanie Hayn.

Given the high concentrations of porewater total soluble sulfides and sediment organic matter in WFH's Middle Harbor, and apparent light limitation in both the Middle and Outer Harbor basins of WFH, I can expect that seagrasses in WFH face multiple environmental stressors, all of which occur as a direct or indirect result of anthropogenic nitrogen enrichment. My research indicates that, in order to protect WFH's seagrasses from further mortality and improve sediment condition, water quality must first be improved.

References

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenyloxidase in beta vulgaris. *Plant Physiology*, *24*(1), 1–15.

Barillé, L., Robin, M., Harin, N., Bargain, A., & Launeau, P. (2010). Increase in seagrass distribution at Bourgneuf Bay (France) detected by spatial remote sensing. *Aquatic Botany*, *92*(3), 185-194.

Boschker, H. T. S., Wielemaker, A., Schaub, B. E. M., & Holmer, M. (2000). Limited coupling of macrophyte production and bacterial carbon cycling in the sediments of Zostera spp. meadows. *Marine Ecology Progress Series*, *203*, 181-189.

Böttcher, M. E., Brumsack, H. J., & Dürselen, C. D. (2007). The isotopic composition of modern seawater sulfate: I. Coastal waters with special regard to the North Sea. *Journal of Marine Systems*, *67*(1-2), 73-82.

Burdige, D. J. (2007). Preservation of organic matter in marine sediments: controls, mechanisms, and an imbalance in sediment organic carbon budgets? *Chemical reviews*, *107*(2), 467-485.

Brodersen, K. E., Nielsen, D. A., Ralph, P. J., & Kühl, M. (2015). Oxic microshield and local pH enhancement protects *Zostera muelleri* from sediment derived hydrogen sulphide. *New Phytologist*, *205*(3), 1264–1276.

de Boer, W. F. (2007). Seagrass–sediment interactions, positive feedbacks and critical thresholds for occurrence: a review. *Hydrobiologia*, *591*(1), 5–24.

Cooper, C. E., & Brown, G. C. (2008). The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. *Journal of bioenergetics and biomembranes*, *40*(5), 533.

Dell'Anno, A., Mei, M. L., Pusceddu, A., & Danovaro, R. (2002). Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. *Marine Pollution Bulletin*, *44*(7), 611-622.

Dennison, W.C. (1990). Chlorophyll content. In *Seagrass Research Methods* (pp. 83–85).

Ferguson, A. J., Eyre, B. D., & Gay, J. M. (2003). Organic matter and benthic metabolism in euphotic sediments along shallow sub-tropical estuaries, northern New South Wales, Australia. *Aquatic Microbial Ecology*, 33(2), 137-154.

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., ... & Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature geoscience*, *5*(7), 505.

Fraser, M. W., & Kendrick, G. A. (2017). Below-ground stressors and long-term seagrass declines in a historically degraded seagrass ecosystem after improved water quality. *Scientific Reports*, *7*(1), 14469.

Frederiksen, M. S., Holmer, M., Borum, J., & Kennedy, H. (2006). Temporal and spatial variation of sulfide invasion in eelgrass (*Zostera marina*) as reflected by its sulfur isotopic composition. *Limnology and Oceanography*, *51*(5), 2308-2318.

Frederiksen, M. S., Holmer, M., Pérez, M., Invers, O., Ruiz, J. M., & Knudsen, B. B. (2008). Effect of increased sediment sulfide concentrations on the composition of stable sulfur isotopes (δ34S) and sulfur accumulation in the seagrasses *Zostera marina* and *Posidonia oceanica*. *Journal of Experimental Marine Biology and Ecology*, *358*(1), 98–109.

Gacia, E., Duarte, C. M., & Middelburg, J. J. (2002). Carbon and nutrient deposition in a Mediterranean seagrass (*Posidonia oceanica*) meadow. *Limnology and Oceanography*, *47*(1), 23-32.

Giblin, A. E., & Howarth, R. W. (1984). Porewater evidence for a dynamic sedimentary iron cycle in salt marshes. *Limnology and Oceanography*, *29*(1), 47-63.

Gilboa-Garber, N. (1971). Direct Spectrophotometric Determination of Inorganic Sulfide in Biological Materials and in Other Complex Mixtures. *Analytical Biochemistry*, *43*, 129–133.

Goodman, J. L., Moore, K. A., & Dennison, W. C. (1995). Photosynthetic responses of eelgrass (*Zostera marina L*.) to light and sediment sulfide in a shallow barrier island lagoon. *Aquatic Botany*, *50*, 37–47.

Groemping, U., & Matthias, L. (2018). Package 'relaimpo'.

Haddad, P. R., & Jackson, P. E. (1990). *Ion chromatography*(Vol. 46). Elsevier.

Hasler-Sheetal, H., & Holmer, M. (2015). Sulfide intrusion and detoxification in the seagrass *Zostera marina*. *PLoS One*, 10(6), e0129136.

Hayn, M. (2012) Exchange of nitrogen and phosphorus between a shallow estuary and coastal waters. M.S. Dissertation, Cornell University

Hayn, M., Howarth, R., Marino, R., Ganju, N., Berg, P., Foreman, K. H., … McGlathery, K. (2014). Exchange of Nitrogen and Phosphorus Between a Shallow Lagoon and Coastal Waters. *Estuaries and Coasts*, *37*(1), 63–73.

Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of paleolimnology*, *25*(1), 101-110.

Hemminga, M. A. (1998). The root/rhizome system of seagrasses: an asset and a burden. *Journal of Sea Research*, *39*(3), 183–196.

Hemminga, Marten A., & Duarte, C. M. C. (2000). *Seagrass ecology*. Cambridge: Cambridge University Press.

Hines, M. E., Knollmeyer, S. L., & Tugel, J. B. (1989). Sulfate reduction and other sedimentary biogeochemistry in a northern New England salt marsh. *Limnology and Oceanography*, *34*(3), 578-590.

Höffle, H., Thomsen, M. S., & Holmer, M. (2011). High mortality of *Zostera marina* under high temperature regimes but minor effects of the invasive macroalgae *Gracilaria vermiculophylla*. *Estuarine, Coastal and Shelf Science*, *92*(1), 35-46.

Holmer, M. (2019). Productivity and biogeochemical cycling in seagrass ecosystems. In *Coastal Wetlands* (pp. 443-477). Elsevier.

Holmer, M., & Hasler-Sheetal, H. (2014). Sulfide intrusion in seagrasses assessed by stable sulfur isotopes—a synthesis of current results. *Frontiers in Marine Science*, *1*, 64.

Holmer, M., & Kendrick, G. A. (2013). High sulfide intrusion in five temperate seagrasses growing under contrasting sediment conditions. *Estuaries and Coasts*, *36*(1), 116-126.

Holmer, M., & Nielsen, S. L. (1997). Sediment sulfur dynamics related to biomass-density patterns in Zostera marina (eelgrass) beds. *Marine Ecology Progress Series*, *146*, 163-171.

Howarth, R. W., & Teal, J. M. (1979). Sulfate reduction in a New England salt marsh1. *Limnology and Oceanography*, *24*(6), 999-1013.

Howarth, R.W., Giblin, A., Gale, J., Peterson, B. J., & Luther III, G. W. (1983). Reduced Sulfur Compounds in the Pore Waters of a New England Salt Marsh. *Ecological Bulletins*, 35, 135–152.

Howarth, Robert W. (1984). The ecological significance of sulfur in the energy dynamics of salt marsh and coastal marine sediments. *Biogeochemistry*, *1*(1), 5–27.

Howarth, R. W., Anderson, D. B., Cloern, J. E., Elfring, C., Hopkinson, C. S., Lapointe, B., ... & Walker, D. (2000). Issues in ecology: Nutrient pollution of coastal rivers, bays, and seas.

Howarth, Robert W., Hayn, M., Marino, R. M., Ganju, N., Foreman, K., McGlathery, K., … Walker, J. D. (2014). Metabolism of a nitrogen-enriched coastal marine lagoon during the summertime. *Biogeochemistry*, *118*(1), 1–20.

Howes, B., Kelley, S. W., Ramsey, J. S., Samimy, R., Schlezinger, D., & Eichner, E. (2006). Linked Watershed-Embayment Model to Determine Critical Nitrogen Loading Thresholds for West Falmouth Harbor, Falmouth, Massachusetts: Massachusetts Estuaries Project, Massachusetts Department of Environmental Protection. Boston, MA.

Jørgensen, B. B., & Revsbech, N. P. (1985). Diffusive boundary layers and the oxygen uptake of sediments and detritus. *Limnology and oceanography*, 30(1), 111-122.

Kiage, L. M., & Walker, N. D. (2009). Using NDVI from MODIS to monitor duckweed bloom in Lake Maracaibo, Venezuela. *Water resources management*, *23*(6), 1125-1135.

Koch, E. W., Ackerman, J. D., Verduin, J., & van Keulen, M. (2007). Fluid dynamics in seagrass ecology—from molecules to ecosystems. In *Seagrasses: biology, ecology and conservation* (pp. 193-225). Springer, Dordrecht.

Kraal, P., Burton, E. D., & Bush, R. T. (2013). Iron monosulfide accumulation and pyrite formation in eutrophic estuarine sediments. *Geochimica et Cosmochimica Acta*, *122*, 75-88.

Lamers, L. P. M., Govers, L. L., Janssen, I. C., Geurts, J. J., Welle, V. der, Ew, M., … Smolders, A. J. (2013). Sulfide as a soil phytotoxin—a review. *Frontiers in Plant Science*, *4*.

Luther III, G. W., Giblin, A., Howarth, R. W., & Ryans, R. A. (1982). Pyrite and oxidized iron mineral phases formed from pyrite oxidation in salt marsh and estuarine sediments. *Geochimica et Cosmochimica Acta*, *46*(12), 2665-2669.

Mangold, K., Shaw, J. A., & Vollmer, M. (2013). The physics of near-infrared photography. *European Journal of physics*, *34*(6), S51.

Marvin-DiPasquale, M. C., Boynton, W. R., & Capone, D. G. (2003). Benthic sulfate reduction along the Chesapeake Bay central channel. II. Temporal controls. *Marine Ecology Progress Series*, 260, 55-70.

McGlathery, K. J., Reynolds, L. K., Cole, L. W., Orth, R. J., Marion, S. R., & Schwarzschild, A. (2012). Recovery trajectories during state change from bare sediment to eelgrass dominance. *Marine Ecology Progress Series*, *448*, 209-221.

Mochida, K., Hano, T., Onduka, T., Ito, K., & Yoshida, G. (2019). Physiological responses of eelgrass (*Zostera marina*) to ambient stresses such as herbicide, insufficient light, and high water temperature. *Aquatic Toxicology*, *208*, 20-28.

Myneni, R. B., Hall, F. G., Sellers, P. J., & Marshak, A. L. (1995). The interpretation of spectral vegetation indexes. *IEEE Transactions on Geoscience and Remote Sensing*, *33*(2), 481-486.

Nelson, W. G. (2017). Development of an epiphyte indicator of nutrient enrichment: Threshold values for seagrass epiphyte load. *Ecological indicators*, *74*, 343-356.

Nixon, S. W. (1995). Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia*, *41*(1), 199–219.

Nixon, S., Buckley, B., Granger, S., & Bintz, J. (2001). Responses of very shallow marine ecosystems to nutrient enrichment. *Human and Ecological Risk Assessment: An International Journal*, *7*(5), 1457-1481.

Ralph, P. J., Durako, M. J., Enriquez, S., Collier, C. J., & Doblin, M. A. (2007). Impact of light limitation on seagrasses. *Journal of Experimental Marine Biology and Ecology*, *350*(1-2), 176-193.

Rees, C. E., Jenkins, W. J., & Monster, J. (1978). The sulphur isotopic composition of ocean water sulphate. *Geochimica et Cosmochimica Acta*, *42*(4), 377-381.

Short, F.T., McKenzie, L.J., Coles, R.G., Vidler, K.P., Gaeckle, J.L. 2006. SeagrassNet Manual for Scientific Monitoring of Seagrass Habitat, Worldwide edition. University of New Hampshire Publication. 75 pp.

Teasdale, P. R., Batley, G. E., Apte, S. C., & Webster, I. T. (1995). Pore water sampling with sediment peepers. *TrAC Trends in Analytical Chemistry*, *14*(6), 250-256.

Terrados, J., Duarte, C. M., Kamp-Nielsen, L., Agawin, N. S. R., Gacia, E., Lacap, D., … Greve, T. (1999). Are seagrass growth and survival constrained by the reducing conditions of the sediment? *Aquatic Botany*, *65*(1), 175–197.

Van Katwijk, M. M., Bos, A. R., Hermus, D. C. R., & Suykerbuyk, W. (2010). Sediment modification by seagrass beds: Muddification and sandification induced by plant cover and environmental conditions. *Estuarine, Coastal and Shelf Science*, *89*(2), 175-181.

Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., ... & Kendrick, G. A. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the national academy of sciences*, *106*(30), 12377-123

Sampling period	Sites: Basin (Site #)	Field collection	Variables measured
July 2017	Outer Harbor (215, 207, 209*, 204*), Middle Harbor (101, 102, 103*, 104*), Snug Harbor (SH-N, SH-C, SH-S)	Seagrass sampling Sediment cores	Shoot density, leaf area Total sulfur, organic matter (LOI)
July 2018—sediments & seagrass	Outer Harbor (204*, 215, 207, 209*), Middle Harbor (101, 102, 103*, 104*)	Seagrass samples Sediment cores	Shoot density, leaf area, hyperspectral photography (NDVI), aboveground vs. belowground biomass ratio, epiphyte biomass Total sulfur, total carbon, organic matter (LOI)
July 2018—peepers	Outer Harbor (215, 209*), Middle Harbor (101), Snug Harbor (SH-S)	Porewater peeper deployment	Total soluble sulfide, sulfate, chloride
August 2018	Outer Harbor (215, 209, 207), Middle Harbor (100, 101, 102), Outside WFH (Buzzards Bay (1,2), Little Island)	Seagrass samples Sediment cores	Hyperspectral photography (NDVI), aboveground vs. belowground biomass ratio Organic matter (LOI)
July 2005-2018	Middle Harbor (101, 102, 103*, 104*), Outer Harbor (204*, 207, 209*, 215, 213, 215, 211, 210), Snug Harbor [prior to 2010] (SH-C, SH-N, SH-S, SH4, SH7, SH9)	Seagrass samples	Leaf tissue $\delta^{34}S$, shoot density, leaf area, synoptic surveys (see text, pg. 7)
July 2007	Snug Harbor (vegetated and unvegetated, near SH-C)	Porewater peeper deployment	Total soluble sulfides

*Table 1: a breakdown of sampling times, sites, and data collected and analyzed during this study. Sites with * symbol occur in between basins near the channel but are grouped with the basin to which they are closest. Only sites from the 2017-2018 sampling period are shown on map (Fig. 3).*

Depth	Parameter	Snug Harbor	Middle Harbor	Outer Harbor
0-2	Organic matter (July 2018)	9.7 (0.8)	14.1 (1.9)	5.4 (5.4)
	Total sulfur (2017)	0.83 (0.1)	0.71 (0.2)	0.23 (0.1)
	Total solid-phase sulfur (2018)	0.91 (0.2)	1.00 (0.1)	0.40 (0.3)
	Total carbon	4.1 (0.3)	6.1 (1.0)	2.2 (2.2)
	Porewater sulfide	0.04	2.0	0.20
	SO42-:Cl- molar ratio	0.052	0.039	0.047-0.047
2-4	Organic matter	9.0 (1.3)	13.5 (2.2)	4.9 (5.6)
	Total sulfur (2017)	0.84 (0.1)	0.98 (0.1)	0.14 (0.1)
	Total solid-phase sulfur (2018)	0.96 (0.1)	1.03 (0.2)	0.47 (0.4)
	Total carbon	3.7 (0.4)	5.8 (1.8)	0.6 (0.1)
	Porewater sulfide	0.20	2.2	0.32
	SO42-:Cl- molar ratio	0.046	0.037	0.046-0.046
4-6	Organic matter	8.2 (1.2)	13.1 (2.1)	4.9 (4.9)
	Total sulfur (2017)	0.83 (0.1)	0.84 (0.2)	0.33 (0.4)
	Total solid-phase sulfur (2018)	0.90 (0.3)	1.08 (0.1)	0.43 (0.2)
	Total carbon	3.3 (1.2)	5.9 (1.2)	1.8 (1.8)
	Porewater sulfide	0.42	2.4	0.43
	SO42-:Cl- molar ratio	0.046	0.036	0.047-0.045
6-8	Organic matter	7.3 (4.2)	12.5 (2.0)	4.7 (2.7)
	Total sulfur (2017)	1.0 (0.1)	0.98 (0.2)	0.17 (0.1)
	Total solid-phase sulfur (2018)	0.82 (0.4)	1.02 (0.1)	0.67 (0.2)
	Total carbon	3.1 (1.7)	5.4 (1.0)	1.6 (0.9)
	Porewater sulfide	0.58	2.3	0.88
	SO42-:Cl- molar ratio	0.045	0.036	0.053-0.042
8-10	Organic matter	8.0 (0.5)	8.5 (3.2)	5.6 (5.0)
	Total sulfur (2017)	0.84 (0.2)	0.84 (0.3)	0.09 (0.0)
	Total solid-phase sulfur (2018)	0.90 (0.1)	0.81 (0.4)	0.68 (0.2)
	Total carbon	3.5 (0.4)	3.4 (1.5)	2.1 (2.0)
	Porewater sulfide	0.68	2.2	0.97
	SO42-:Cl- molar ratio	0.042	0.037	0.046-0.039
10-12	Organic matter	8.6 (0.7)	4.2 (2.4)	4.7 (4.0)
	Total sulfur (2017)	0.90 (0.2)	0.64 (0.2)	0.12 (0.0)
	Total solid-phase sulfur (2018)	1.1 (0.1)	0.59 (0.4)	0.46 (0.3)
	Total carbon	3.7 (0.4)	1.6 (1.1)	1.8 (1.8)
	Porewater total soluble sulfides (mM)	0.88	2.0	0.96
	SO42-:Cl- molar ratio	0.038	0.035	0.045-0.040

*Table 2**:** Sediment parameters by basin and depth. Values in ( ) indicate one standard deviation. Note that there are two porewater sites in the Outer Harbor (Site 209 and 215). For porewater sulfide and sulfate to chloride molar ratio, the first listed value in the Outer Harbor column is site 209, while the second is site 215.*

Site	Region	Organic matter (% by weight) Core avg. 2017	Organic matter (% by weight) Core avg. 2018	Total C (% by weight) Core avg. 2018	Total S (% by weight) Core avg. 2017	Total solid-phase S (% by weight) Core avg. 2018	Porewater sulfide (µM) Rooting zone avg. (1-8 cm) 2018	Porewater sulfide (µM) Peeper avg. (1-14 cm) 2018	SO_4^{2-}:Cl^- molar ratio Peeper avg. (1-14 cm) 2018
207	Outer Harbor	2.7 (0.8)	2.1 (0.7)	0.7 (0.2)	0.2 (0.1)	0.4 (0.3)	--	--	--
209	Outer Harbor	1.5 (0.4)	2.4 (0.4)	0.9 (0.3)	0.1 (0.1)	0.4 (0.2)	52.9 (31.0)	625.5 (527.1)	0.047 (0.004)
215	Outer Harbor	7.1 (1.4)	10.0 (1.5)	3.7 (1.0)	0.4 (0.2)	0.7 (0.2)	441.7 (266.3)	896.1 (382.3)	0.042 (0.004)
100	Middle Harbor	--	7.2 (1.1)*	2.9 (1.4)*	--	0.7 (0.2)*	--	--	--
101	Middle Harbor	11.9 (3.2)	12.6 (3.4)	5.5 (2.0)	0.8 (0.1)	1.1 (0.2)	2319.3 (187.1)	1929.6 (578.6)	0.037 (0.004)
102	Middle Harbor	12.6 (3.8)	10.8 (4.6)	4.7 (2.2)	0.9 (0.3)	0.8 (0.3)	--	--	--
Snug Harbor		10.9 (1.3)	8.4 (1.8)	3.6 (0.8)	0.9 (0.1)	0.9 (0.2)	385.4 (266.5)	736.7 (352.6)	0.044 (0.007)
Outside WFH		--	0.45 (0.3)*	0.1 (0.0)*	--	0.2 (0.1)*	--	--	--

*Table 3: Sediment and porewater variables at selected sites, integrated over depth. Reported as: "value (1 standard deviation)". West Falmouth Harbor 2018 data. A -- symbol represents that there is no data for that region, variable, site, or time period. All samples taken during July sampling period, except where indicated with a * symbol, in which case samples were retrieved in early August.*

		NDVI July—August	Chlorophyll content (μg/cm^2)	Aboveground: belowground biomass ratio (July)	Aboveground: belowground biomass ratio (August)	2005-2011 avg. leaf tissue sulfur isotopic composition ($\delta^{34}S$)	2013-2017 avg. leaf tissue sulfur isotopic composition ($\delta^{34}S$)	Epiphyte cover (mg epiphyte DW per cm^2 leaf area)	Epiphyte cover (mg epiphyte DW per g leaf DW)	Average Aboveground biomass (g DW per m^2) July	Average Belowground biomass (g DW per m^2) July
207	Outer Harbor	0.02-0.09	15.3 (5.7)	3.0 (0.5)	2.9 (0.4)	+1.2 (6.0)	+6.8 (10.0)	0.50 (0.31)	65.0 (12.7)	365	121
209	Outer Harbor	0.03-0.09	10.3 (4.8)	3.7 (0.1)	2.3 (0.5)	+15.1 (1.9)	+10.7 (6.2)	0.52 (0.20)	56.2 (3.3)	294	80
215	Outer Harbor	0.06-0.07	12.1 (6.5)	4.4 (0.2)	3.4 (1.0)	+10.0 (6.3)	+8.4 (5.6)	0.90 (0.25)	83.6 (23.0)	362	83
100	Middle Harbor	-- - 0.08	8.9 (4.4)	--	3.1 (0.4)	--	--	--	--	--	--
101	Middle Harbor	0.08-0.12	18.3 (7.4)	5.2 (1.0)	2.8 (0.4)	--	+2.0 (2.7)	0.81 (0.14)	111.1 (15.5)	355	69
102	Middle Harbor	0.09-0.11	13.3 (4.3)	7.5 (5.7)	3.1 (0.5)	--	-0.1 (2.6)	0.72 (0.15)	52.2 (19.3)	650	87
Snug Harbor		--	--	--	--	+0.2 (4.8)	--	--	--	--	--
Outside WFH		-- - 0.09	9.7 (3.7)	--	2.1 (0.5)	--	--	--	--	--	--

Table 4: A compilation of seagrass-health variables that we collected in and around West Falmouth Harbor 2017-2018 at select sites. Values in ( ) indicate one standard deviation, while a - - symbol indicates a lack of data for that site, region, or time period.